The Synthesis of α-Tocohexaenol, a New Fluorescent Analogue of α-Tocopherol

by

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Abstract

Since its discovery in 1922, vitamin E has been widely investigated for its role as a powerful, chain-breaking antioxidant that is required for human health. However, some basic issues still remain unclear, such as the mechanism and dynamics of the intracellular trafficking of α-tocopherol. To better understand tocopherol's biological activity at the cellular level, fluorescence spectroscopy and microscopy have been found to be valuable tools.

This thesis reports the synthesis of a new fluorescent analogue of α-tocopherol, α-tocohexaenol, an intrinsically fluorescent analogue of α-tocopherol. Different methodologies of preparation have been attempted and a strategy using a preformed chromanol head plus C_{10} and C_5 portion of the polyene side chain finally provided us the desired α-tocohexaenol. α-Tocohexaenol shows a strong fluorescence in both ethanol and hexanes with maximum λ_{ab} = 368 nm and maximum λ_{em} = 521 nm. This compound is stable for a couple of weeks in ethanol or hexane solution if stored at 0 °C and protected form light. It decomposes slowly at room temperature and light will accelerate its decomposition (within 5 hours). Thus, α-Tocohexaenol may be a useful fluorescent probe to study the biochemistry and cell biology of vitamin E.

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List of Abbreviations

Ac	acetyl
AVED	ataxia with isolated vitamin E deficiency
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMF	N,N-dimethylformamide
DIBAL-H	Diisobutyl aluminum hydride
FAB	fast atom bombardment
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IR	infrared spectrometry
LAH	lithium aluminum hydride
LHMDS	lithium hexamethyldisilylamide
MS	mass spectrometry
NBS	N-bromosuccinimide
NMR	nuclear magnetic resonance
RT	room temperature
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TTP	tocopherol transfer protein

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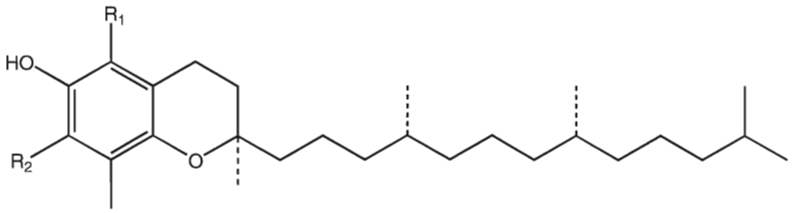
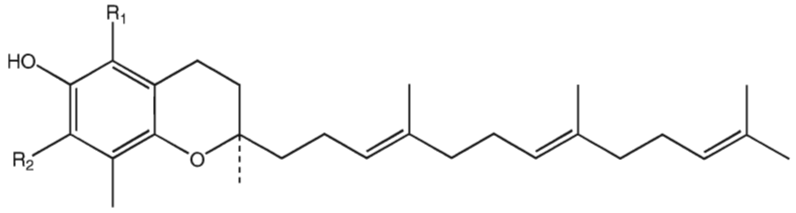
1. Introduction

1.1 Discovery and Structure of Vitamin E

The vitamins are a group of compounds that are required in the diet in small amounts for the maintenance of normal health and metabolic integrity. Vitamin E was discovered in 1922 by Herbert Evans and Katherine Bishop in green leafy vegetables.[1] In 1924 Barnett Sure named it vitamin E.[2] It was scientifically named tocopherol because vitamin E supported fertility. This word comes from the Greek word *tokos* which means childbirth, and *phero* meaning to bring birth, and the *ol* referring to the alcohol properties of this molecule. At present vitamin E is regarded as a generic name for four tocopherols and four tocotrienols that have the following general structural features: an aromatic chromanol head and a 16-carbon hydrocarbon tail. The difference between tocopherols and tocotrienols is that tocopherol has a saturated side chain derived from phytyldiphosphate (PDP), while tocotrienol has an unsaturated isoprenoid side chain derived from geranylgeranyldiphosphate (GGDP).[3] Tocopherols and tocotrienols are further separated into individual compounds assigned by prefix α, β, γ, δ, depending on the number and position of methyl substitution on the chromanol ring (Figure 1).



Tocopherol



Tocotrienol

	R_1	R_2
α-Tocopherol/Tocotrienol	CH_3	CH_3
β-Tocopherol/Tocotrienol	CH_3	H
γ-Tocopherol/Tocotrienol	H	CH_3
δ-Tocopherol/Tocotrienol	H	H

Figure 1. Chemical structures of tocopherols and tocotrienols.

Vitamin E is an essential component of the human diet and is synthesized exclusively by photosynthetic organisms.[3] Tocopherols are present in vegetable oils and in the germ of cereal seeds, whereas tocotrienols are the primary form of vitamin E in the seed endosperm of most monocots, including cereal grains such as wheat, rice, barley, and palm oil. Vitamin E vitamers have different bioactivity as shown in Table 1.[4, 5] The biological activity of the major forms of vitamin E are based upon the 'fetal resorption-gestation' method in rats, an assay that determines the ability of various forms of vitamin

E to maintain live fetuses in pregnant rats.[6] α-Tocopherol was established as the most active tocopherol in preventing the death of rat embryos.

	Fetal Resorption Bioassay		
	IU/mg	**Relative Activity**	**Binding to α-TTP**
D-α-tocopherol (*RRR*)	1.36	1.0	1.0
D-β-tocopherol (*RRR*)	0.75	0.50	0.38
D-γ-tocopherol (*RRR*)	0.15	0.10	0.09
D-δ-tocopherol (*RRR*)	0.05	0.03	0.02
D- α-tocotrienol	0.75	0.50	0.12
D- β-tocotrienol	0.08	0.05	-
D- γ-tocotrienol	-	-	-
D- δ-tocotrienol	-	-	-

Table 1. Relative biological activity[7] and affinity to α-TTP (α-tocopherol transfer protein) of tocopherols and tocotrienols (all racemic α-tocopherol acetate = 1 IU/mg).[4]

1.2 Biosynthesis of Tocopherols and Tocotrienols

Tocopherols and tocotrienols are synthesized in plants via two converging pathways as shown in Figure 2.[8-10] The aromatic part of the chromanol ring is derived from the precursor homogentisic acid that is formed by *p*-hydroxyphenylpyruvate dioxygenase (HPD) catalyzed oxygenation of *p*-hydroxyphenylpyruvate, a tyrosine metabolite. HPD catalyzes the most complex reaction in the pathway: oxidative decarboxylation of the pyruvate side chain to acetate; 1,2-migration of the acetate chain; and hydroxylation of C-1 of the aromatic ring to yield the homogentisic acid.[11] The second ring of the chromanol head (the pyran) is partly derived from the side chain.

The prenyl side chain is formed from the condensation of dimethylallylpyrophosphate (DMAPP) and three units of IPP to form geranylgeranylpyrophosphate (GGPP). GGPP is reduced to phytylpyrophosphate by GGPP reductase.[12] Homogentisate and phytylpyrophosphate are fused together by homogentisate prenyltransferase (HPT) to give 2-methyl-6-phytylplastoquinone, the common precursor to all tocopherols.[13, 14] Addition of the saturated phytyl chain leads to tocopherols, addition of the unsaturated chain leads to tocotrienols.[15] 2-Methyl-6-phytylplastoquinone can directly be cyclized by tocopherol cyclase to yield δ-tocopherol.[16] The transfer of a methyl group from *S*-adenosylmethionine to the 3-position of the aromatic ring yields 2,3-dimethyl-6-phytylplastoquinone, which is cyclized to yield γ-tocopherol. Final transfer of a methyl group to the 5-position of the chromanol ring catalyzed by γ-tocopherol methyl transferase converts γ- and δ-tocopherol into α- and β-toxcopherol respectively.[17] Tocotrienols are formed by a corresponding pathway.[8, 15]

1.3 Absorption and Transport of Vitamin E

All forms of vitamin E are absorbed as the free phenol form by the intestine without discrimination of the individual isomers.[18] The esterified forms of α-tocopherol that are commonly present in dietary supplements are hydrolyzed by the pancreatic carboxyl ester hydrolase in the intestine before absorption.[19, 20] The absorption of vitamin E is relatively poor, only 20% to 40% of a dose is normally absorbed from the small intestine.[19, 21]

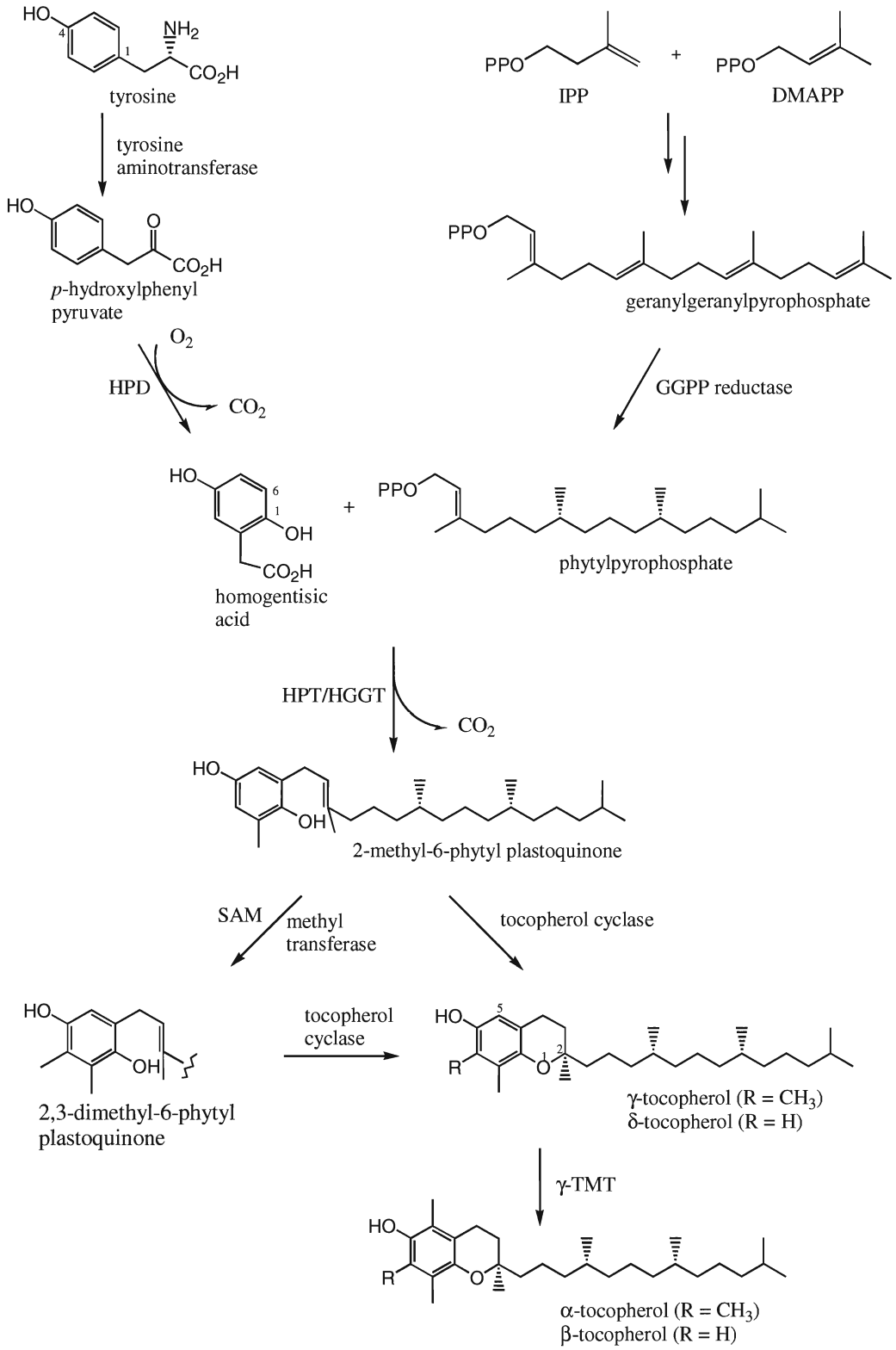


Figure 2. Biosynthesis of tocopherols and tocotrienols (IPP-isopentenyl pyrophosphate; DMAPP-dimethylallylpyrophosphate; HPD-*p*-hydroxyphenylpyruvate dioxygenase; GGPP-geranylgeranylpyrophosphate; HPT-homogentisate prenyltransferase; HGGT-homogentisate geranylgeranyl transferase; SAM-*S*-adenosylmethionine; TMT-methyltransferase).[8]

In intestinal mucosal cells, all vitamers of vitamin E are incorporated into chylomicrons, and chylomicron remnants are then taken up by the liver.[22] The liver is a major storage site of α-tocopherol, accounting for one-third of the total body content of α-tocopherol.[23] Chylomicron pathway is thought to be predominant for vitamin E absorption in the intestine.[24] However, an alternative pathway for vitamin E absorption via HDL efflux was also suggested by the improvement of vitamin E deficiency due to the use of high dose supplementation in chylomicron-dificient patients.[24] This HDL-dependent mechanism has been characterized by Anwar et al. in $CaCo_2$ cells.[25]

α-Tocopherol, which binds to the liver α-tocopherol transfer protein (α-TTP), is then exported in very low-density lipoprotein (VLDL) and is available for tissue uptake.[26] Later it appears in low-density lipoprotein (LDL) and high-density lipoproteinm (HDL) as a result of metabolism of VLDL in the circulation. The other vitamers, which do not bind well to α-TTP are not incorporated into VLDL;[27, 28] they are metabolized in the liver and excreted.

Lipoproteins are the major carriers of vitamin E. Both LDL and HDL are important sources of vitamin E for cellular uptake.[24] Two mechanisms were suggested for the tissue uptake of vitamin E: lipoprotein lipase releases some of the vitamin E from the chylomicron structure by hydrolyzing the triacylglycerol in chylomicrons and VLDL, whereas separately there is receptor-mediated uptake of LDL-bound vitamin E. Mardones' study suggested that the main mechanism for tissue uptake of vitamin E from plasma lipoprotein is by way of scavenger receptor class B type I (SR-BI).[29]

The binding activity of α-TTP in rat liver cytosol was first reported by Catignani in 1975.[30] In 1995 Arita *et al.* cloned the human transcript encoding TTP.[31] It is generally

accepted that by facilitating the secretion of α-tocopherol from hepatocytes to lipoproteins TTP functions as a key regulator of vitamin E status.[32] The affinity of α-TTP for the vitamers varies: α-tocopherol shows the highest affinity, followed by β-, γ-, and δ-tocopherol.[28] How α-TTP mediates the secretion of α-tocopherol into VLDL is not fully understood. It has been hypothesized that the role of α-TTP is not in the secretion, but in the cytosolic transport of α-tocopherol from late endosome to the plasma membrane.[33]

In liver the metabolism of vitamin E is initiated by cytochrome P450 (CYP450). The ω-oxidation that is the hydroxylation of the ω-methyl group is followed by the removal of carbon units by β-oxidation.[34] Different isoforms of CYP450 enzymes have been suggested to be responsible for the metabolism. The cytochrome P450 isoform CYP4F2 catalyzes tocopherols other than *RRR*-α-tocopherol into water-soluble products that are excreted in urine.[35] Figure 3 shows the final products of tocopherol oxidative metabolism: carboxyethyl-hydroxychromans (CEHC), and its intermediates, carboxymethylhexyl-hydroxychromans (CMHHC) and carboxymethylbutyl-hydroxychromans (CMBHC) .[34]

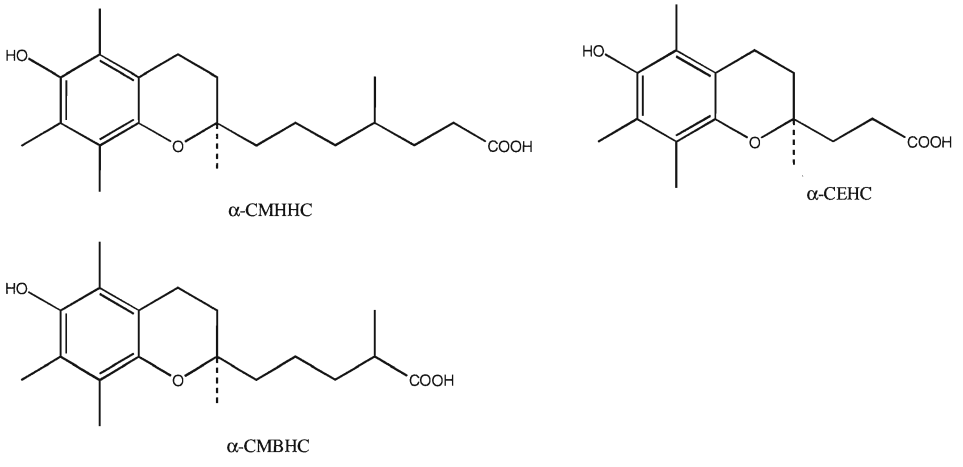


Figure 3. Metabolites of α-tocopherol.

α-Tocopherol has the longest half-life in circulation (48 hours); that of β- and γ- is only of the order of 13 to 15 hours.[5] The retention time of α-tocopherol in tissues varies. In the lungs the vitamin has a half life of 7.6 days, in liver 9.8 days, in skin 23.4 days, in brain 29.4 days, and in the spinal cord 76.3 days.[36]

1.4 Vitamin E as an Antioxidant

Polyunsaturated fatty acids undergo oxidative attack by hydroxyl radicals that yield alkylperoxyl radicals after reaction with oxygen (Figure 4), which perpetuate a chain reaction in the lipid with potentially disastrous consequence for cells.[8, 37] The initiating step of this chain reaction is the abstraction of a hydrogen atom from the bis-allylic site on an unsaturated fatty acid to yield a carbon-centered radical. The hydrogen atom is almost invariably abstracted from a bis-allylic methylene carbon. Once the radical is formed it immediately reacts with molecular oxygen to form a fatty acid peroxyl radical. The fate of the peroxyl radical depends on the following reactions: (A) Encounter with an antioxidant such as vitamin E (chain termination). (B) Encounter with another fatty acid (chain propagation). (C) Encounter with a double bond in the same molecule to form an endoperoxide. (D) Loss of oxygen (β-fragmentation).

Vitamin E is generally regarded as the most important lipid-soluble antioxidant in blood plasma and circulating lipoproteins. It can inhibit lipid peroxidation in biological membranes by scavenging the chain-propagating peroxyl radicals as shown in reaction (1): [38, 39]

$$ROO\cdot + Toc\text{-}OH \rightarrow ROOH + Toc\text{-}O\cdot \quad (1)$$

The resultant chromanoxyl radicals could also participate in further propagation of lipid peroxidation, which is called pro-oxidation:

$$\text{Toc-O}\cdot + \text{RH} \rightarrow \text{Toc-OH} + \text{R}\cdot \qquad (2)$$

The LDL particle has a lipid core consisting of neutral lipids (cholesteryl and triglyceride esters of polyunsaturated fatty acids, and free cholesterol) and a coat of polar lipids (phosphatidylcholine esters of C18:2 and C20:4).[8] When LDL is attacked by a peroxyl radical, the resulting radical will likely react with α-tocopherol to generate a hydroperoxide and the tocopheroxyl radical. Because the life time of this radical is sufficiently long (estimated about 12.5 s), it will eventually abstract a hydrogen from a bis-allylic methylene from linoleic or arachidonic acid, creating a fatty acid radical and a new autoxidation chain reaction.[40] This is called α-tocopherol-mediated peroxidation (TMP). TMP will cause oxidative damage to lipids especially in the absence of coantioxidants such as vitamin C (ascorbate) or ubiquinone.[41, 42]

On the other hand it can be reduced back to vitamin E by reductants, which do not propagate lipid peroxidation:

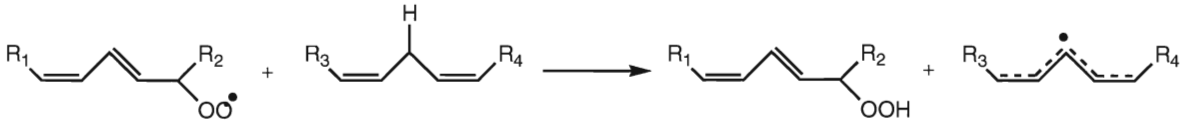
$$\text{Toc-O}\cdot + \text{AH2} \rightarrow \text{Toc-OH} + \text{AH} \qquad (3)$$

The tocopheroxyl radicals can be reduced back to tocopherol by redox-active reagents such as vitamin C or ubiquinol.[43, 44] In homogeneous solution phase autoxidation, the tocopheroxyl radicals will react with a second peroxyl radical to give a non-radical product which leads to the destruction of α-tocopherol as an antioxidant.[8]

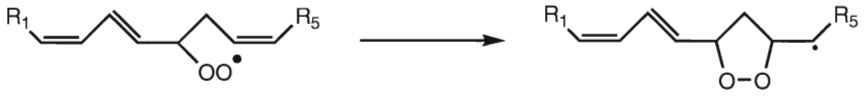
In addition, both α- and γ-tocopherol can react with peroxynitrite which comes from the reaction between nitric oxide and superoxide (Figure 5). γ-Tocopherol is more reactive than α-tocopherol and undergoes nitration.[45]

Formation of fatty acid peroxyl radical & β-fragmentation:

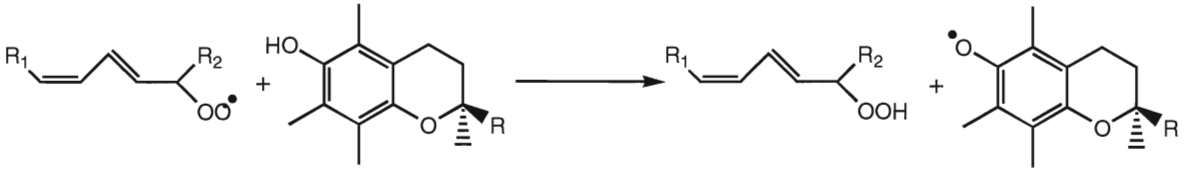
Chain propagation:



Endoperoxide formation:



Termination of the radical reaction by α-tocopherol:



Reduced back to α-tocopherol

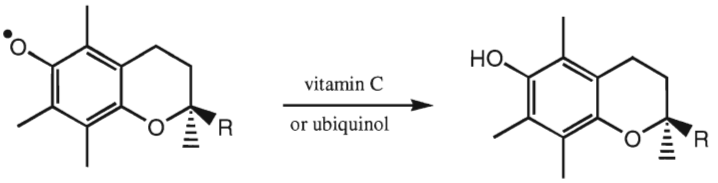


Figure 4. Reactions of fatty acid peroxyl radicals.[8]

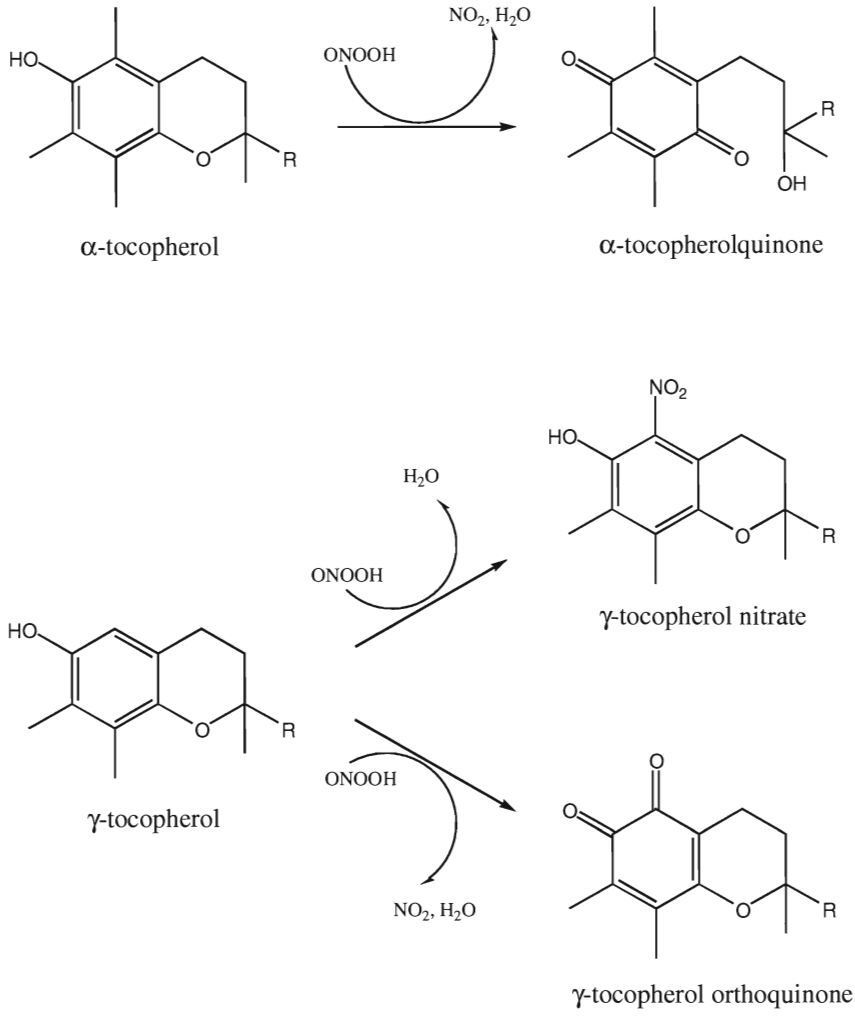


Figure 5. Reaction of α-tocopherol and γ-tocopherol with peroxynitrite.[4]

1.5 Therapeutic Potentials of Vitamin E

1.5.1 Vitamin E Deficiency and Ataxia with Isolated Vitamin E Deficiency

Vitamin E deficient pregnant female animals suffer fetal resorption.[1] Vitamin E deficiency in laboratory animals also leads to muscular dystrophy[46] and neurologic lesions.[47, 48] Combined dietary deficiency of vitamin E and selenium causes fatal myopathy.[49]

Dietary deficiency of vitamin E is rare in humans, however, patients with severe fat malabsorption will develop vitamin E deficiency. Vitamin E deficiency causes severe

spinocerebellar lesions coupled with low plasma vitamin E levels.[50] Patients lacking hepatic α-TTP suffer from "ataxia with isolated vitamin E deficiency" (AVED). Traber *et al.* first suggested that there was a connection between AVED and the dysfunctional incorporation of α-tocopherol into VLDL in the liver.[18] In 1995 it was established that the molecular defects responsible for AVED are mutations in the gene-encoding α-TTP.[50, 51] The extremely low plasma levels of α-tocopherol in AVED patients can be elevated by dietary vitamin E supplementation.[18]

Animal models in which the expression of TTP has been disrupted unequivocally demonstrate the relationship between vitamin E, normal health, and TTP integrity.[32] For example, plasma tocopherol levels are very low in $TTP^{-/-}$ mice.[52] As $TTP^{-/-}$ mice age, they display the neurological symptoms associated with AVED.[53] The severe pathologies caused by vitamin E deficiency in humans also clearly demonstrated that vitamin E is an essential nutrient for human health.[32]

1.5.2 Cardiovascular Disease

The oxidation of biological molecules, such as lipids, proteins and DNA, by molecular oxygen, is regarded to be involved in the development of numerous pathological events, such as cancer, cardiovascular disease (CVD) and even the aging process. Vitamin E, as a chain-breaking antioxidant, will suppress the oxidation and protect biological molecules and tissues from oxidative damage. Therefore, consumption of certain foods enriched in vitamin E is thought to reduce the incidence of CVD and cancer.[54] It was also hypothesized that tocopherol might prevent oxidative modification of LDL, a process believed to be involved in the formation of atherosclerotic plaque.[55]

There is considerable controversy regarding any beneficial effect of vitamin E in preventing CVD,[56] but it should be keep in mind that vitamin E supplementation was found to be beneficial for certain patients in prevention trials.[57-59]

1.5.3 Cancer

α-Tocopherol succinate was the first vitamin analogue that demonstrated the ability to inhibit the growth of cancer cells.[60] This effect has been shown for numerous additional cancer cell lines.[8, 61] Tocotrienols were shown to inhibit the growth of human breast cancer cells in culture.[62, 63] Fuchs' study shows that the risk of colon cancer for women taking folate, through multi-vitamin (including vitamin E) intake for at least 15 years, was reduced 75 %.[64]

Several mechanisms are thought to be involved in vitamin E's anti-cancer effects: the inhibition of lipid peroxidation and the formation of reactive products such as fatty acid peroxyl radicals; direct effects on tumor cells such as control of tumor growth through induction of differentiation; elimination of tumor cells by increased efficacy of antitumor actions by the immune system.[8, 45, 65-67]

There are also negative reports on the relationship between cancer and vitamin E. In Kline's study there is no evidence that vitamin E protects women from breast cancer.[68] Another report suggests that there is no protective effect of α-tocopherol on prostate cancer incidence.[69] The inconsistent results between clinical studies and epidemiological data could arise from using different forms of vitamin E.[70]

1.5.4 Cataracts

There is good evidence that cataracts are the result of oxidative damage to α-crystallin in the lens of the eyes, and therefore dietary intakes of antioxidants might be beneficial.[4]

One study suggested a reduction in the risk of cataracts of at least 50% by taking more supplementary vitamins C and E.[71] Another study that investigated the effect of vitamin E on the development of cataract and age-related maculopathy reported a positive result.[72]

1.6 Vitamin E: non-antioxidant Functions

α-Tocopherol inhibits platelet aggregation both in vitro and in vivo, in response to agonists such as arachidonic aicd and phorbol ester. This seems to be mediated by a protein kinase C dependent mechanism.[4] Activation of protein kinase C is a key step in signal transduction and is involved in cell growth and differentiation.[73] Protein kinase C can be inhibited by physiologic concentration of α-tocopherol in the medium of a variety of cells.[74] Inhibition of protein kinase C may also be the mechanism by which α-tocopherol inhibits vascular smooth muscle proliferation, a factor in thrombus formation and platelet aggregation.[75-77]

In monocytes, α-tocopherol reduces formation of reactive oxygen species, cell adhesion to the endothelium, and release of interleukins and tumor necrosis factor. α-Tocopherol inhibits the assembly of the respiratory burst NADPH oxidase that is responsible for oxygen radical generation. The effects on cell adhesion are from the inhibition of protein kinase C. The effects on cytokine release is from inhibition of 5-lipoxygenase. In macrophages, α-tocopherol reduces release of interleukin-1 and the activity of the scavenger LDL receptor, which in turn reduces the accumulation of cholesterol and transformation into foam cells.[78, 79] In experimental animals, vitamin E deficiency depresses immune system function, which suggests a signaling role of vitamin E in the immune system.[80]

1.7 Tocotrienols: vitamin E beyond tocopherols

As *D*-α-(*RRR*)-tocopherol has the highest biological activity in the vitamin E family; the term vitamin E is often used synonymously with α-tocopherol. Compared to α-tocopherol, the tocotrienols have received much less attention. However, recent studies indicate that tocotrienols possess unique biological functions.[81] Although tocopherols and tocotrienols share similar structure, the unsaturated side chain of tocotrienols allows for more efficient penetration into tissues such as brain and liver.[82] Yoshida et al. reported that the corresponding tocopherols and tocotrienols exerted the same reactivity toward radicals in solution, but tocotrienols were more readily transferred between the membranes and incorporated into membranes than tocopherols.[83] α-Tocotrienol showed much higher antioxidant potency than α-tocopherol against lipid peroxidation in rat liver microsomal membranes; the reasons for this were suggested by Serbinova *et al.* as follows: higher recycling efficiency from chromanoxyl radicals; more uniform distribution in membrane bilayer; stronger disordering of membrane lipids which makes interaction of chromanols with lipid radicals more efficient.[38]

α-Tocotrienol also possesses numerous functions that are not shared by α-tocopherol.[81] For example, nanomolar concentration of α-tocotrienol prevents inducible neurodegeneration by regulating specific mediators of cell death.[84] Tocotrienol administration reduces oxidative protein damage and extends the mean life span of *C. elegans*.[85] Tocotrienol but not tocopherol suppresses the growth of human breast cancer cells.[86]

1.8 Aims & objectives

Deuterated and radiolabeled compounds have been used in the study of the biological activity of vitamin E. However, the study of vitamin E's biokinetics and distribution has been impossible at the cellular level because the above-mentioned method requires tissue extracts, which lacks the time and spatial resolution to determine the rates of vitamin E movement between different pools inside and outside of a cell. So it would be advantageous to use a non-destructive technique such as fluorescence spectroscopy-microscopy. Vitamin E is only weakly fluorescent, therefore in order to use fluorescence spectroscopy-microscopy a fluorophore should be attached to the molecule. Using this strategy a series of fluorescent analogues of α-tocopherol have been synthesized in our lab[87] and successfully used in the study of binding with TTP and intracellular trafficking of vitamin E.[88, 89] Another analogue was synthesized by Oleynik *et al.* as an off/on fluorescent antioxidant indicator (Figure 6) .[90]

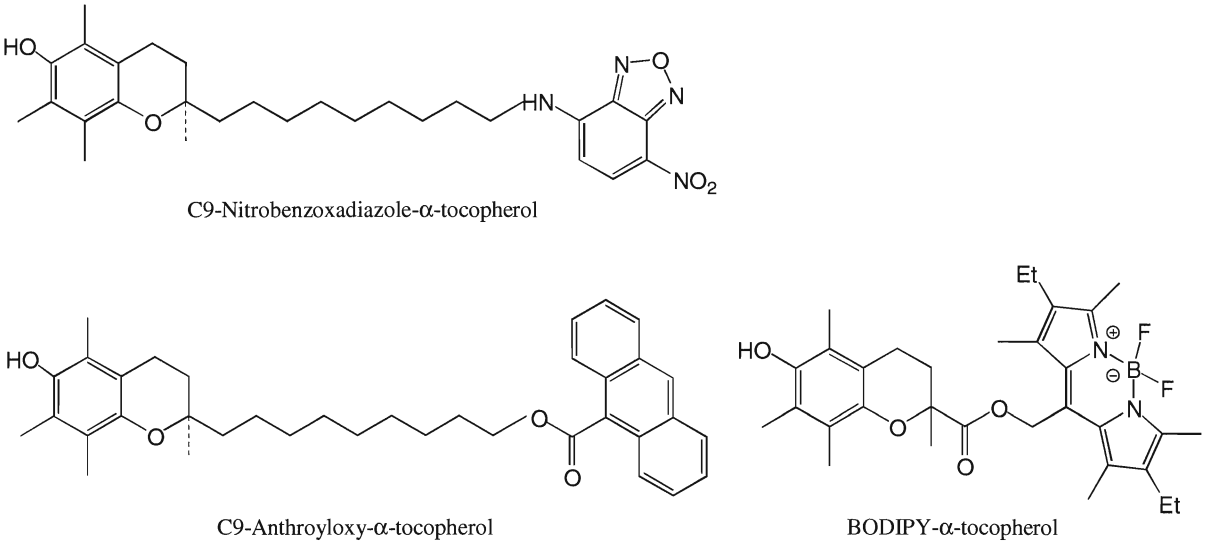
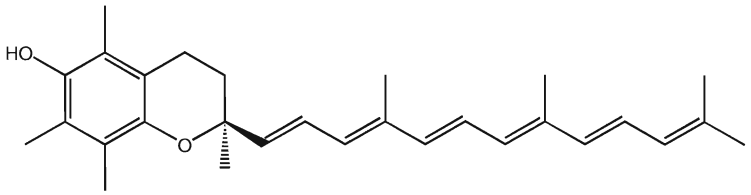


Figure 6. Structure of three fluorescent analogues of α-tocopherol.

However such fluorescent analogues of α-tocopherol may also change the structure of the α-tocopherol to a degree that affects its ability to act like the natural ligand (α-tocopherol). For instance, NBD-α-tocopherol is appreciably more water-soluble than α-tocopherol. A new fluorescent analogue of α-tocopherol was designed and synthesized in this thesis (Figure 7).



Compound **1** α-tocohexaenol

Figue 7. Structure of α-tocohexaenol **1**.

This new molecule, α-tocohexaenol, keeps much of the structure of α-tocopherol: it has the same chromanol head and the skeleton of α-tocopherol. The only difference is that six conjugated double bonds are introduced to its side chain, which will make it fluorescent. In addition, by combing a chromanol head of α-tocopherol and a conjugated polyene system similar to carotenoids, it could be a more potent antioxidant itself.[91, 92] This new compound is expected to show a fluorescence excitation maximum of 366 nm (calculated according to Fieser-Kuhn rules), and an emission maximum of 465 nm. Compared with α-tocopherol, which has an excitation maximum of 292 nm and emission maximum of 325 nm, α-tocohexaenol's fluorescent properties will make it a useful fluorescent reporter that is not interfered with by endogenous fluorophores such as tryptohan.

A great deal of evidence exists to show that α-tocopherol has considerable conformational freedom in a phospholipid membrane.[93] It may bob up and down to some degree; it can rotate about its long axis; and it is able to diffuse within the two-dimentional fluid of one leaflet of the bilayer.[93] We would predict that the *trans*-polyene

tail of α-tocohexaenol will make it more rigid so that it can not have as much conformational freedom as α-tocopherol when residing in membranes. Figure 8 is a cartoon illustration of α-tocohexaenol in a membrane. Since α-tocopherol will spend some time in a fully extended conformation, α-tocohexaenol will mimic the activity of α-tocopherol to some extent.

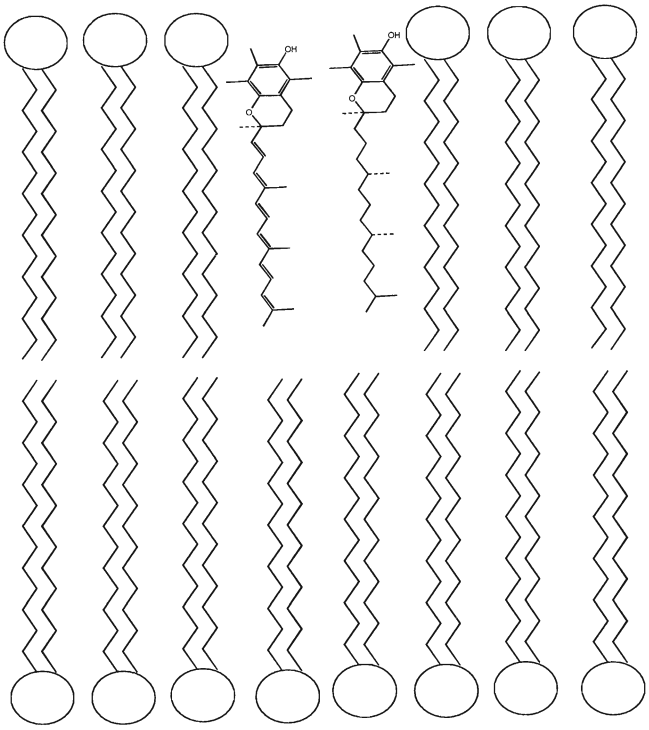


Figure 8. Cartoon illustration of α-tocohexaenol & α-tocopherol in a phospholipid membrane.

2. Results and Discussions

2.1 Design of a fluorescent analogue of Vitamin E

In order to use a non-destructive method such as fluorescence spectroscopy in the study of the bioactivity of vitamin E, a suitable analogue is necessary. To design new fluorescent analogues of α-tocopherol the following requirements must be considered:

1) It should disturb the structure of the α-tocopherol as little as possible.

2) It must have good fluorescent characteristics.

α-Tocohexaenol **1** (Figure 8) is considered to meet these requirements and will have a λ_{abs} > 300 nm. According to Fieser-Kuhn rules[94] the absorption wavelength of this conjugated polyene compound can be calculated as follows:

$\lambda_{max} = 114 + 5 \times 25 + 6(48.0 - 1.7 \times 6)$

$= 366$ nm

Based on the similarity to a comparable system used by Prestwich *et al.*[95] it is expected to have a fluorescent emission maximum of 465 nm, which will be distinguishable from the background fluorescence of a cell.

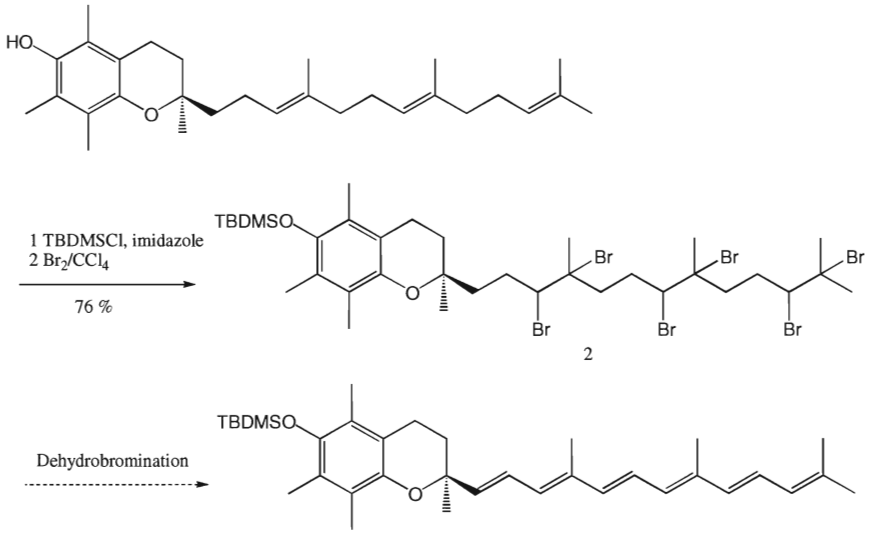
2.2 Synthesis of Compound 1

2.2.1 α-Tocotrienol as Starting Material

Since the only difference between our target compound and α-tocotrienol is that the target molecule has a conjugated side chain with six double bonds, whereas α-tocotrienol has a side chain with three methylene-separated double bonds, starting from α-tocotrienol appeared to be a possible choice. α-Tocotrienol is available in gram scale from palm kernel oil Tocomin-50® by column chromatographic purification. To introduce a

conjugated system to the side chain we have tried several different chemistries. All our efforts on converting α-tocotrienol to α-tocohexaenol **1** will be discussed in the following section.

2.2.1.1 Bromination-dehydrobromination Method



Scheme 1 Bromination/dehydrobromination of a-tocotrienol.

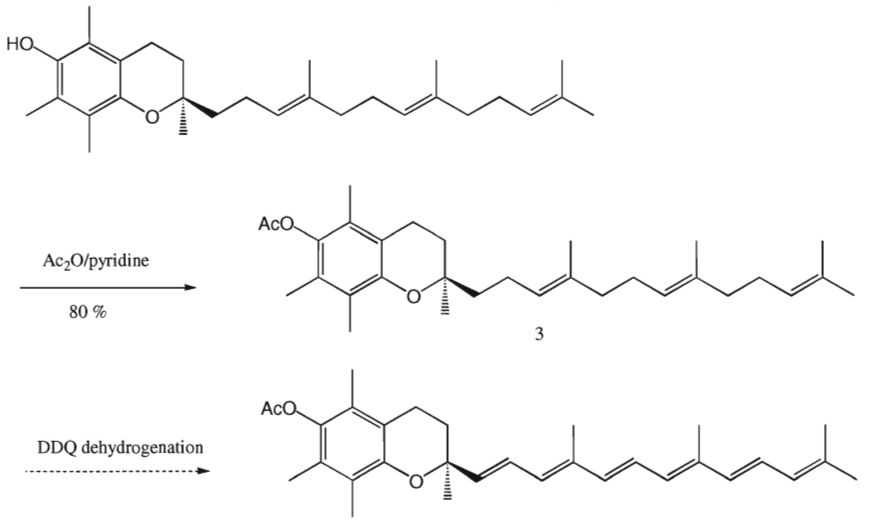
Kuklev and Smith[96] reported the successfully transformation of 1,4,7-octatriene methylene interrupted *cis*-double bonds of naturally occurring polyunsaturated fatty acid to a polyconjugated 1,3,5,7-octatetraenes using bromination-dehydrobromination. In their method the bromination of α-linolenic aicd (ALA) with bromine molecule yielded dibromides, then treatment with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) initiated didehydrobromination and yielded a mixture of conjugated isomers; base catalyzed *cis*-trans isomerization finally yielded the all-*trans* product (Schme 2).

The same strategy was tried on α-tocotrienol. First the phenol group of α-tocotrienol was protected as the TBDMS ether in the presence of imidazole, then the bromination with 3

molar equivalent of bromine molecule in CCl_4 successfully transformed the a-tocotrienol to its hexabromide product. Unfortunately, treatment with various different bases in different solvents including sodium hydride or potassium *tert*-butoxide in dry THF, n-butyllithium in dry ether, sodium hydroxide in ethanol, sodium ethoxide in ethanol, and silver oxide in ethyl acetate did not give any desired product (for details please see the thesis of Gu Fan[97]). We also tried such extreme conditions as heating the hexabromide with potassium hydroxide without solvent but again no product was obtained. This method was therefore abandoned.

2.2.1.2 DDQ Dehydrogenation Method

DDQ (2,3-dichloro-5,6-dicyano-*p*-benzoquinone) is a good hydrogen acceptor in dehydrogenations forming double bonds adjacent to conjugated double bonds or aromatic rings.[98-100] We hoped that treatment with DDQ would form the more stable conjugated system and would be the driving force to dehydrogenate α-tocotrienol.



Scheme 2 DDQ dehydrogenation route to a-tocohexaenol.

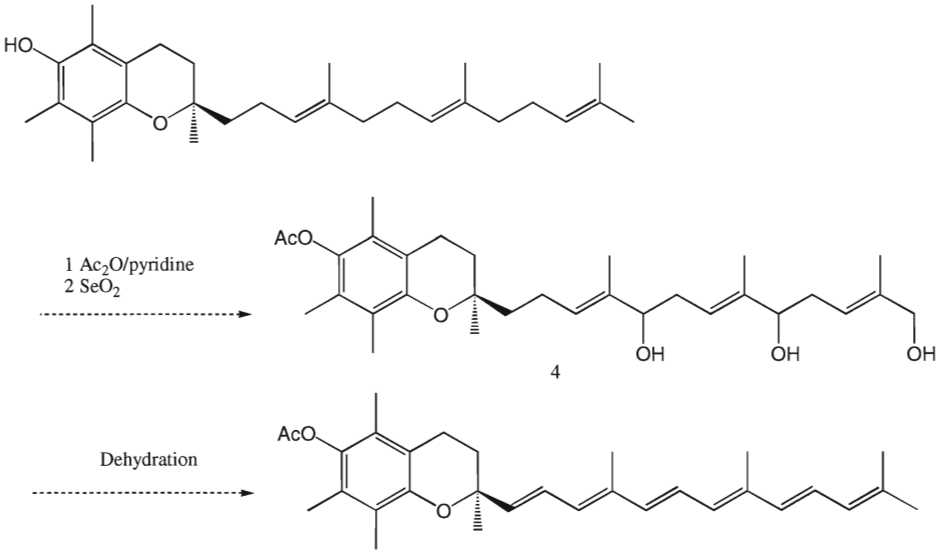
Scheme 2 illustrates this DDQ dehydrogenation pathway. The phenol group of α-tocotrienol was first protected as the acetate ester by treatment with acetic acid anhydride in pyridine, then the mixture of acetate ester protected α-tocotrienol and DDQ in toluene was heated in a sealed tube for various times. Unfortunately, only starting material was recovered (30-50%). When the molar equivalent of DDQ was increased from 3 to 10, all starting material was destroyed and small amounts of products were obtained. Although the mass spectrum showed that there was a weak peak of interest (m/z = 458) which corresponds to the molecular ion of the desired product, the HPLC analysis of the final product showed that it was a complex mixture. More importantly, there was no absorption around 360 nm which is the characteristic absorption of six conjugated double bonds. Thus this method was abandoned as well.

2.2.1.3 Allylic-hydroxylation by Selenium Dioxide and Dehydration Method

Selenium dioxide is used in the allylic hydroxylation of isolated double bonds in straight-chain hydrocarbons which transform an allylic methylene to an allylic alcohol. Considering the difficulty of removing colloidal selenium and the formation of organoselenium by-products, M.A. Umbreit and K.B. Sharpless[101] suggested a method in which *tert*-butyl hydroperoxide (TBHP) was used as a re-oxidant to reoxidize the reduced selenium species to SeO_2, so the reaction can proceed with catalytic amount of SeO_2. In our experiment the Sharpless method was followed.

The oxidation reaction went very slowly, even after 48 hours of reaction most starting material was recovered (80-90%). When the molar equivalent of TBPH was increased to 7.2, two polar products were obtained. No big improvement was observed when the molar equivalent of TBPH was further increased to 14.4. From the TLC two polar spots

other than the starting material were visualized. However, we could not determine their structure even with its NMR, IR and MS spectra. Considering its poor yield (~10%), we chose another hydroxylation reagent: osmium tetroxide.

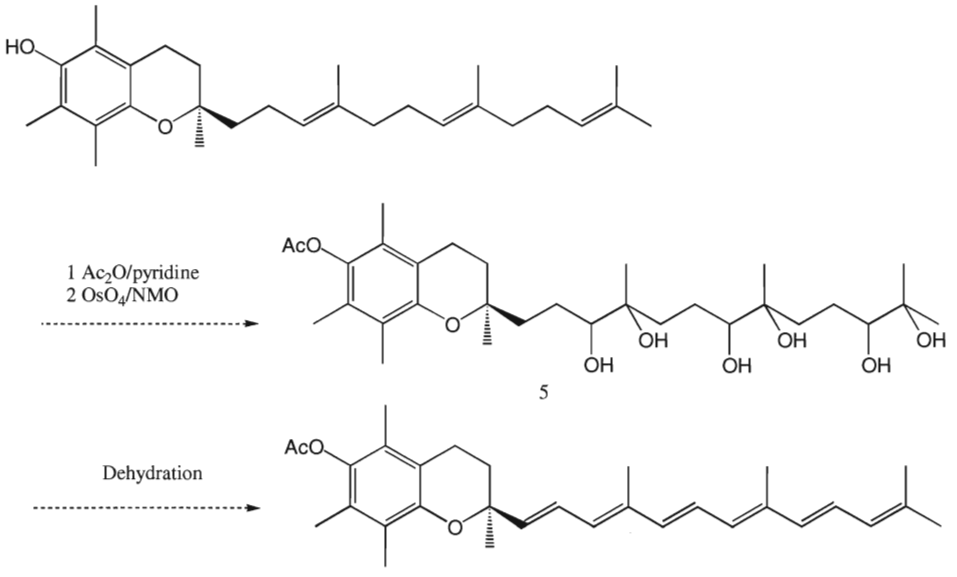


Scheme 3 Allylic-hydroxylation/dehydration of a-tocotrienol.

2.2.1.4 Osmium Tetroxide Dihydroxylation and Dehydration Method

Oxidization of an olefin with osmium tetroxide is the most reliable way for *cis*-dihydroxylation of a double bond.[102] The reaction of an olefin with a stoichiometric quantity of osmium tetroxide in pyridine, followed by reductive hydrolysis, is a common procedure. However, in consideration of the high cost and toxicity of osmium tetroxide, and the difficult workup procedure when pyridine is used, new procedures using catalytic amounts of osmium tetroxide were developed. For example, Hofmann's procedure relies on metal chlorates as the stoichiometric oxidant,[103] and Milas' procedure relies on hydrogen peroxide.[104] However, these two reagents give rise to over-oxidation products

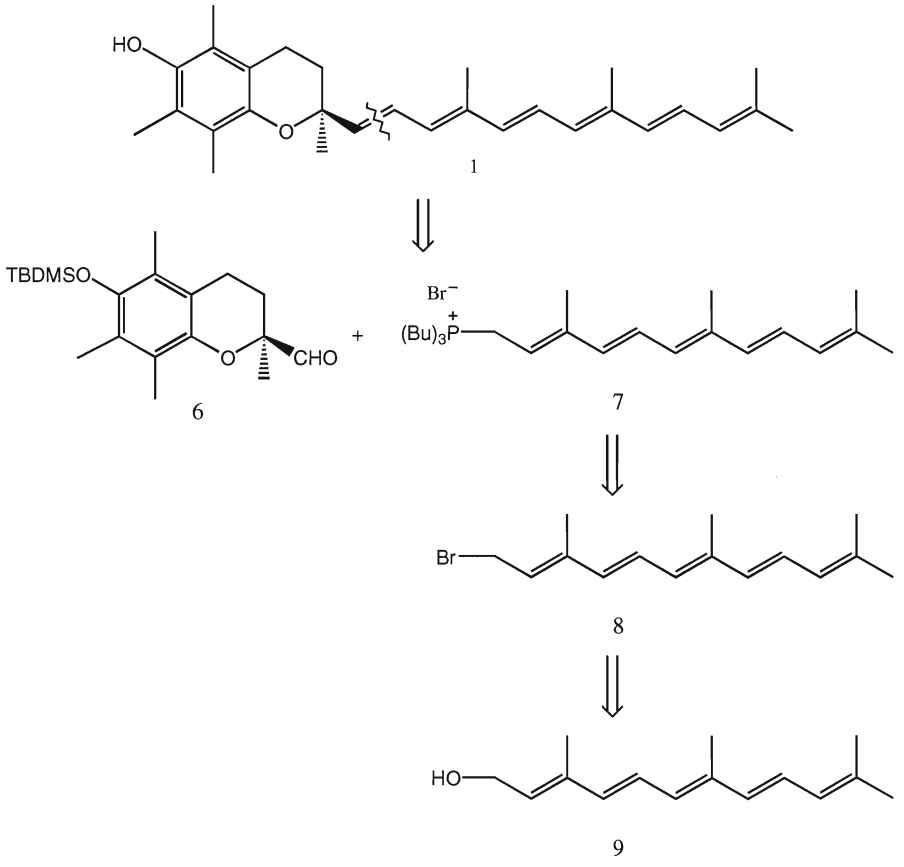
(ketols and compounds resulting from cleavage of the C-C bond). To suppress the by-products, Sharpless developed a procedure that used 0.2 % equivalent of OsO_4, 1.6 equivalent of *tert*-butyl hydroperoxide and 10% equivalent of Et_4NOH.[105] Unfortunately Sharpless' method did not give us any hydroxylation product. Even after 72 hours reaction, only starting material was recovered (30-40%). We also tried the procedure which used NMO (N-methyl-morpholine-N-Oxide) as the stoichiometric oxidant[102] but once again no product was obtained except starting material. Sharpless also reported negative result with tri- and tetrasubstituted olefins.[106] They found that olefins which failed to react with catalytic OsO_4 were potent inhibitors of the catalytic process. These olefins apparently trapped the osmium by forming an extremely stable osmate ester. That probably explained why we did not observe any reaction in our experiments.



Scheme 4 Osmium tertaoxide oxidation/dehydration of a-tocotrienol.

Literature reports describing the di-hydroxylation of more than two double bonds are rare. There is only one article that reported the successful synthesis of the hydroxyl analogue of α-tocopherol.[107] Considering their poor yield (~0.5 %) and the uncertainty of the next dehydration step, we chosed to synthesize compound **1** through the multi-step synthesis outlined in Scheme 5.

2.2.2 Synthesis α-Tocohexaenol using Wittig Chemistry



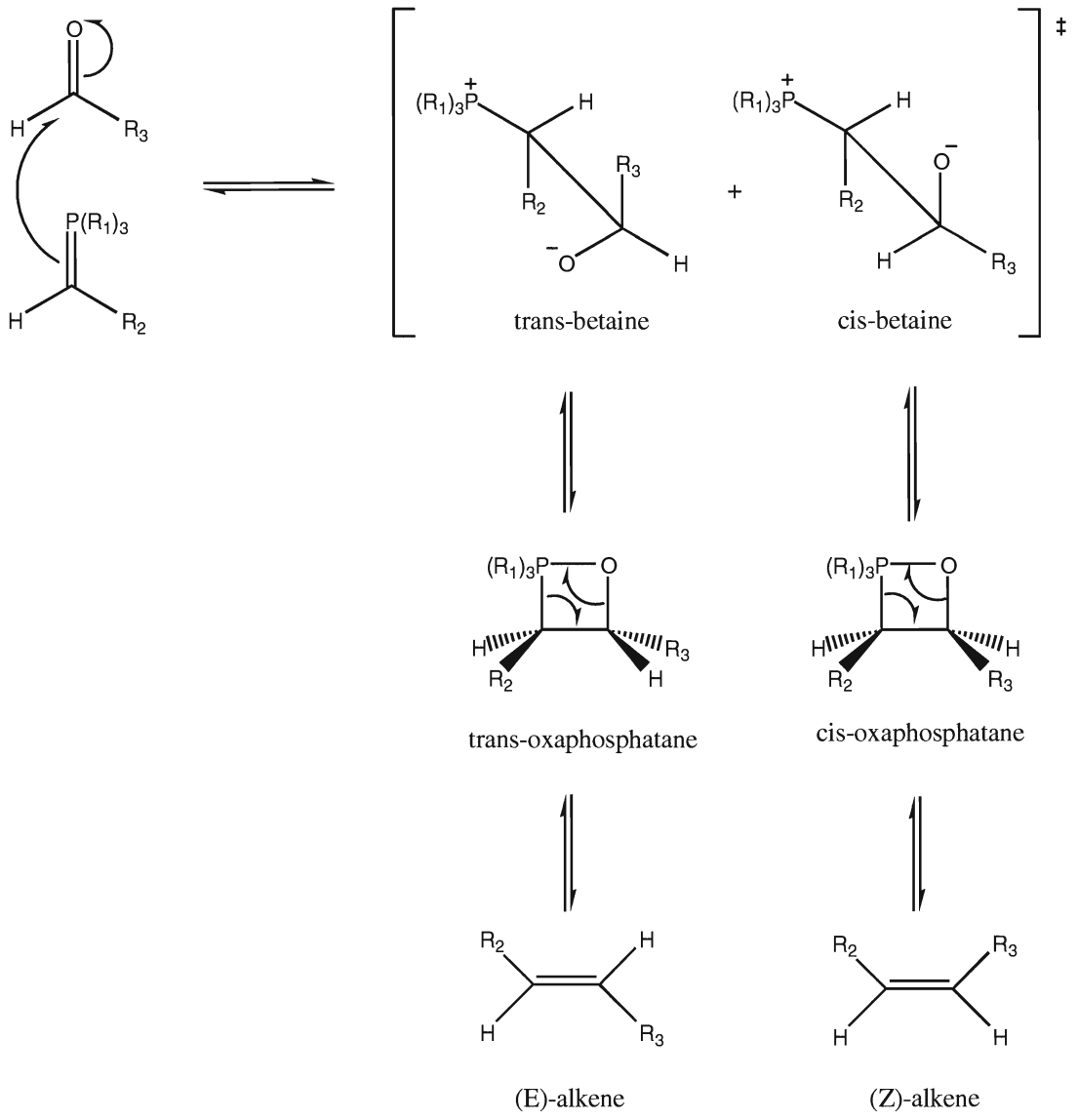
Scheme 5 Retrosynthesis of compound **1** based on the use of phosphonium salt.

2.2.2.1 Mechanism of Wittig Reaction

The Wittig reaction is one of the most important and the most effective methods for synthesis of carbon-carbon double bonds.[108] The active reagent in this transformation is the phosphorous ylide, which is usually prepared from a triaryl- or trialkylphosphine and an alkyl halide followed by deprotonation with a suitable base. The mechanism of the Wittig reaction is shown in Scheme 6. First, the nucleophilic attack of the ylide to the carbonyl group leads to a betaine. The steric bulk of the ylide influences the stereochemical outcome of nucleophilic addition to give a predominance of the *cis*-betaine, which then forms the cis-oxaphosphatane. Elimination gives the *Z*-alkene and triphenylphosphine oxide. Carbon-carbon bond rotation of the *cis*-betaine gives the trans-betaine, which leads to *E*-alkene through a *trans*-oxaphosphatane. However, the existence and interconversion of the betaine is still under debate. There is evidence that the oxaphosphatanes can be formed directly from the [2+2] cycloaddition of phosphonium ylides and carbonyl compounds.[109]

There are three different types of ylides depending on the nature of R_2 substitute: stabilized ylides, when R_2 is a strong electron withdrawing group that stabilizes the negative charge on the carbon; semi-stabilized ylides, where R_2 is an aryl or alkenyl substitute which is less stabilizing; nonstabilized ylides, when R_2 is an alkyl substitute which do not stabilize the negative charge on the carbon.

The stereochemistry of the olefination of aldehyde with phosphorous ylide is governed basically by the nature of the ylide. Nonstabilized ylides give predominantly *Z*-alkenes; while stabilized ylides give predominantly *E*-alkenes; semi-stabilized ylides usually give a mixture of *E* and *Z* isomers.

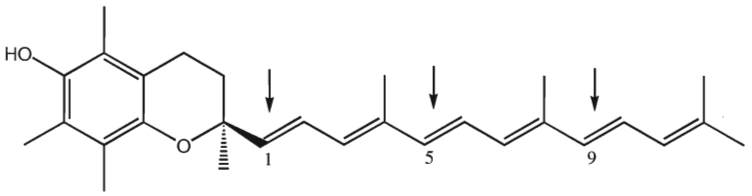


Scheme 6. Mechanism of Wittig reaction.[110]

The stereoselectivity is also influenced by many other factors, such as the ligands at phosphorous, the nature of solvent and the presence of metal ions. For example, less bulky ligands such as triethyl or tributyl phosphonium salts, favor *E*-alkene when semi-stabilized ylides were used.[111, 112] For non-stabilized ylides the stereoselectivity of the reaction is largely independent of the solvent polarity.[113] Stabilized ylides can be made *cis*- or *trans*-selective according to the solvent used in the reaction. Under Boden's

conditions (with a catalytic amount of 18-crown-6 ether) the stereochemical result is significantly dependent on the type of solvent and the nature of the ylide.[114] In the case of Wittig reactions with semi-stabilized ylides, the presence of lithium ion leads to a product mixture which is enriched with *Z* alkene; while when sodium or potassium ions are present, the mixture is enriched with *E* alkene.[115]

2.2.2.2 Synthesis of Compound 1 using C_{14} + C_{15} Strategy



Compound **1** α-tocohexaenol

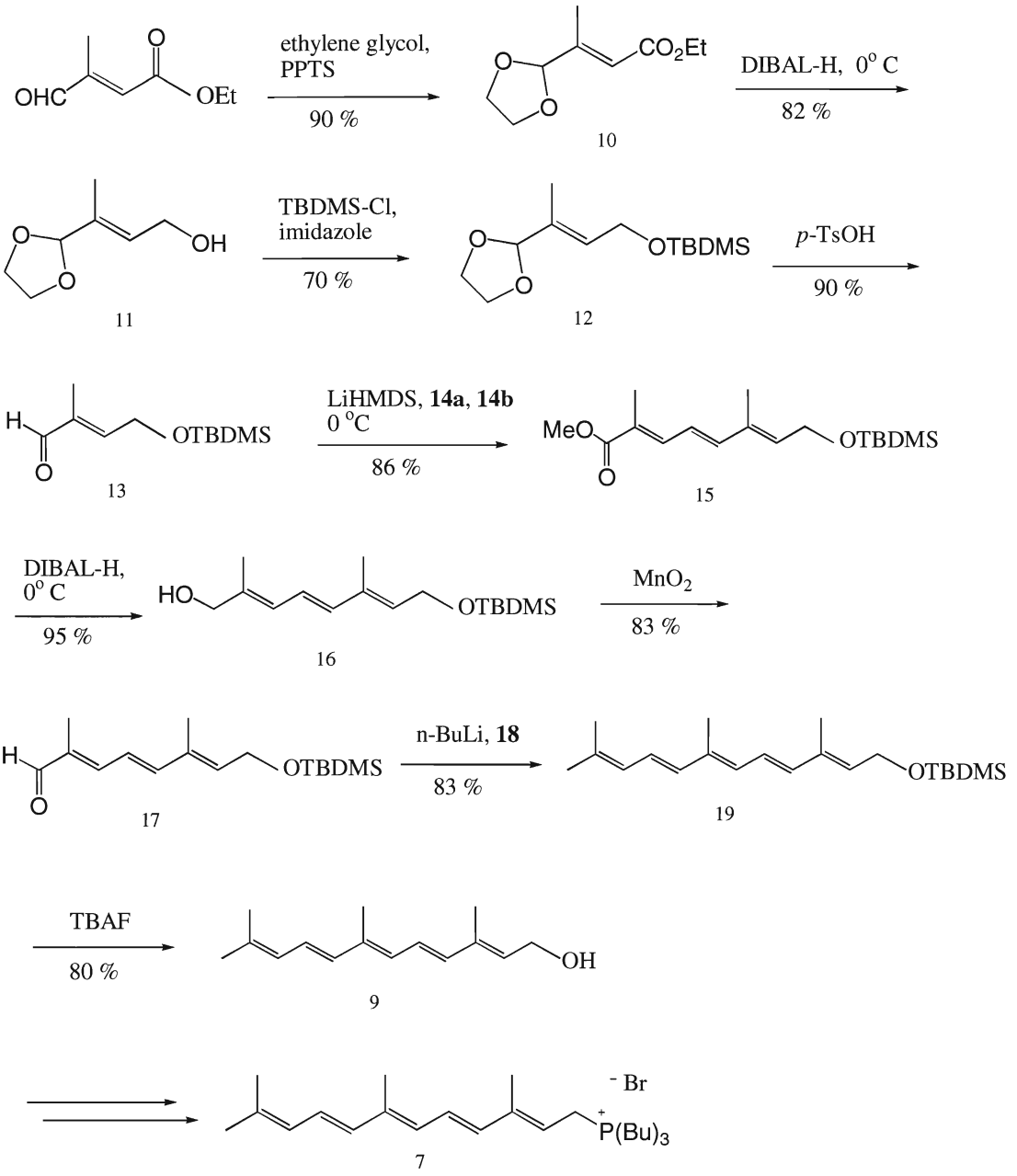
Figure 9. The positions of three additional double bonds in compound **1** as compared to α-tocopherol.

In preparing all-*trans* α-tocohexaenenol **1,** the 1*E*, 5*E* and 9*E* double bonds were stereoselectively installed through Wittig olefination. Scheme 7 illustrates our first effort to synthesize compound **1** using Wittig olefinations. The synthesis of pentaenol **9** was reported by Liu and Prestwich.[95] We hoped that the pentaenol **9** can be converted to its bromide product **8** via a substitution reaction then treated with a trialkyl phosphine to give the phosphonium salt **7**, which would then be coupled with Trolox aldehyde **6** to yield the final product **1**.

The all-*trans* alcohol **9** was prepared according to Prestwich's method as outlined in Scheme 7. Starting from ethyl (*E*)-3-formylbut-2-enoate, the aldehyde group was first protected as an acetal (ethylene glycol, PPTS, benzene, 91%). Reduction of ester **10** with

lithium aluminum hydride at 0 °C afforded alcohol **11** (70 %). The protection of alcohol **11** as silyl ether **12** (TBDMSCl, imidazole, DMF, rt, 90 %) was followed by deprotection of the acetal (*p*-TsOH, H_2O, 90%) to give the required aldehyde **13**. The resulting aldehyde **13** was coupled with allylic diethylphosphonate **14a** through Horner-Wadsworth-Emmons reaction to give all-*trans* triene ester **15** (84 %).

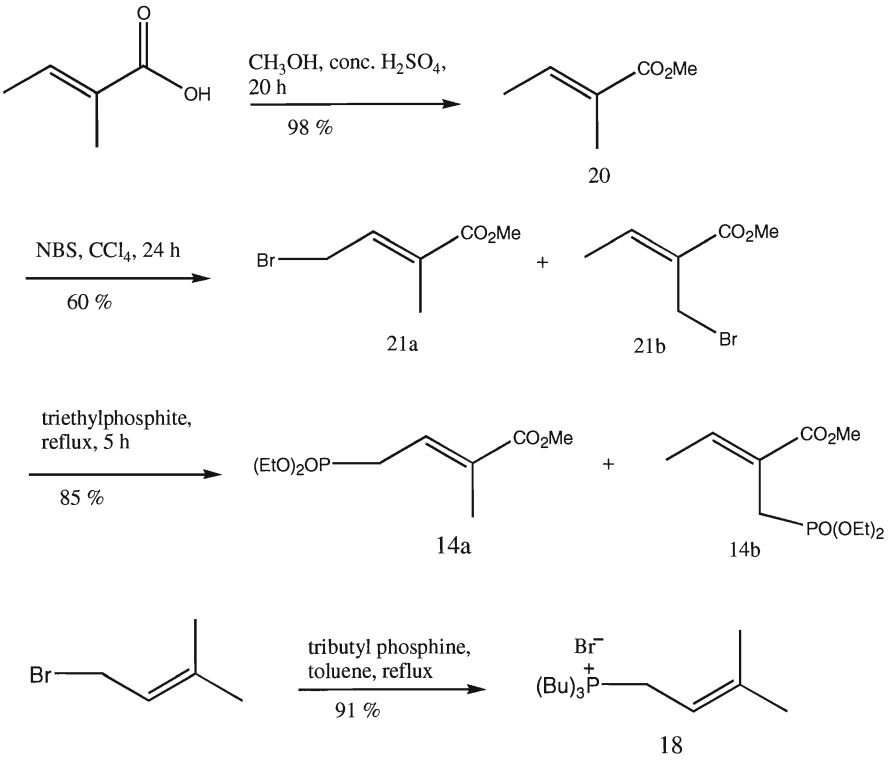
The phosphonate esters **14a** and **14b** were prepared from tiglic acid as outlined in Scheme 8. First, the allylic bromination of tiglic methyl ester **20** by NBS in CCl_4 gave a mixture of bromide **21a** and **21b** at 2:1 ratio. The ratio of the two isomers was determined by their ^{1}H NMR. The vinyl proton of **21a** had a chemical shift at 6.93 ppm as a quartet, whereas the vinyl proton of **21b** had a chemical shift at 7.13 ppm and appeared as a triplet. The following Michealis-Arbuzov reaction (Scheme 8) gave a mixture of **14a** and **14b** in a 3:2 ratio (based on ^{1}H NMR). The mixture of **14a** and **14b** was used in the Horner-Wittig reaction without separation. Only **14a** was coupled with aldehyde **13** in the Horner-Wadsworth-Emmons reaction to give the triene product **15**. The reason for this selectivity is probably due to the fact that **14a** is less sterically hindered than **14b** so it is selectively consumed in the reaction. n-Butyl lithium was first used as the base to deprotonate **14a** to generate the corresponding ylide, as described by Prestwich. However it only gave a poor yield (~ 20 %). We presumed that n-butyl lithium was consuming most of the aldehyde before it coupled with the ylide. Therefore we tried less nucleophilic bases, such as potassium *t*-butoxide and LHMDS; the later one gave an excellent yield (84 %).



Scheme 7 Synthesis of alcohol **9**.

Subsequent reduction of ester **15** with DIBAL-H at 0 °C gave alcohol **16** (88 %), which was then oxidized by active MnO_2 to yield aldehyde **17** (80 %). We also examined the use of Dess-Martin reagent in this oxidation step, which gave a similar yield (76 %). Considering the ease of use and the lower cost, we chose MnO_2 as the oxidizing reagent

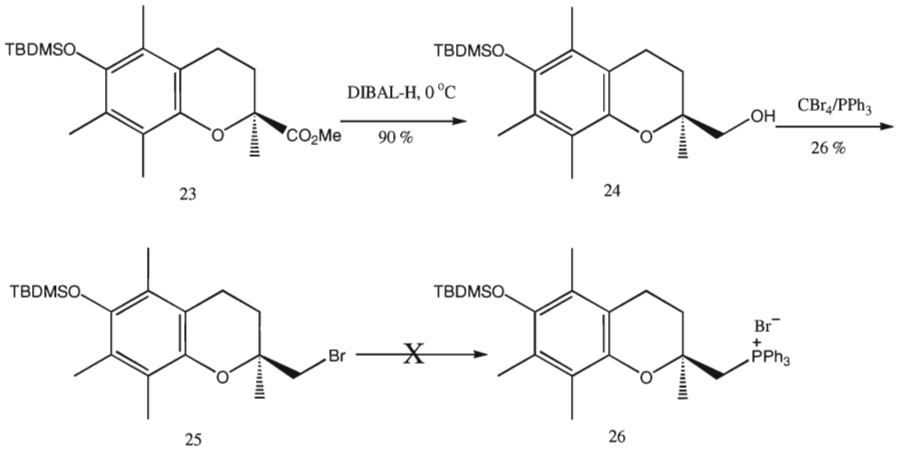
in the end. The resulting aldehyde **17** was coupled with Wittig salt **18**, which was derived from 1-bromo-3-methylbut-2-ene (Scheme 8), to give pentaene **19**. Deprotection of the silyl ether gave pentaenol **9**. However, all our efforts to transform the pentaenol **9** to its



Scheme 8 Synthesis of phosphonate ester **14a**, **14b** and Wittig salt **18**.

Wittig salt failed. Treatment of alcohol **9** with halogenation reagents, such as HBr, PBr_3, or CBr_4 and PPh_3, followed by reacting with PPh_3 or PBu_3, did not provide the desired phosphonium salt. Treatment of alcohol **9** with triphenylphosphine hydrobromide only gave a very poor yield (10 %) despite the results of Furuhata *et al.* who reported a one-pot Wittig reaction which used a similar conjugated allylic alcohol as starting material.[116] In our case treatment of alcohol **9** with triphenylphosphine hydrobromide followed by adding aldehyde **6** and KOH in methanol gave no condensation product. In all our efforts

only aldehyde **6** was recovered. We believed that the bromide **8** or the phosphonium salt **7** was too labile and that they decomposed quickly before they could be treated with a base, although reports on the instability of Wittig salts with a conjugated polyene system are rare. We also considered the possibility of switching the moiety bearing the phosphonium salt. However the Trolox bromide derived from Trolox® did not even react with triphenylphosphine, even when refluxed in toluene for 5 days (Scheme 9), presumably due to the sterically congested nature of this position.



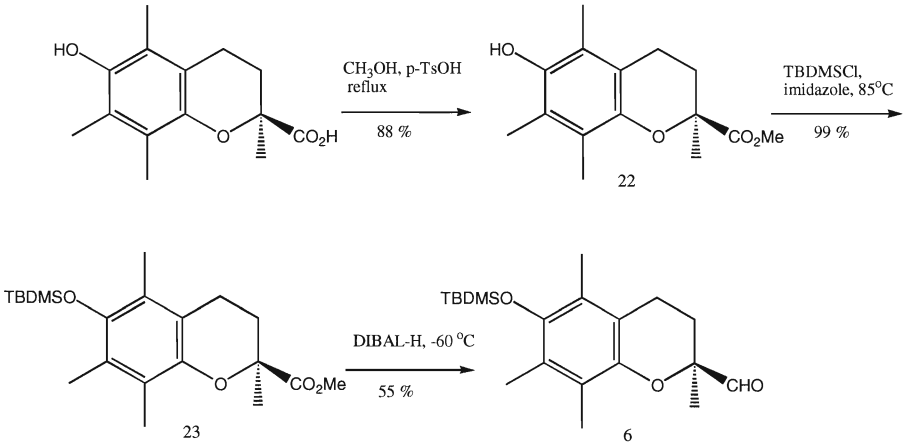
Scheme 9 Synthesis of Trolox phosphonium salt.

2.2.2.3 Synthesis of Compound 1 using $C_{14} + C_{10} + C_5$ Strategy

Instead of coupling the Trolox aldehyde **6** with Wittig salt **7**, the aldehyde **6** was coupled with Wittig salt **28** first, with the rationale that by lacking one conjugated double bond it will be more stable than its pentaene analogue **7** (Scheme 11).

Deprotection of silyl ether **15** by TBAF in THF gave alcohol **27** (65 %). However, the conversion of alcohol **27** to bromide **28** was not efficient. Several bromination reagents were used such as hydrobromide acid solution, PBr_3 and CBr_4 with PPh_3, but the best

yield was only 40 %. When directly treated with triphenylphosphine hydrobromide, alcohol **27** was successfully converted to Wittig salt **28** with excellent yield (88 %). The Wittig salt was then deprotonated by LHMDS to give the corresponding ylide that was coupled with aldehyde **6** to give ester **29**. Reduction of ester **29** with DIBAL-H at 0 °C afforded **30** (70 %), which was oxidized by active MnO_2 to yield all-*trans* aldehyde **31**, that was used in the next Wittig reaction.

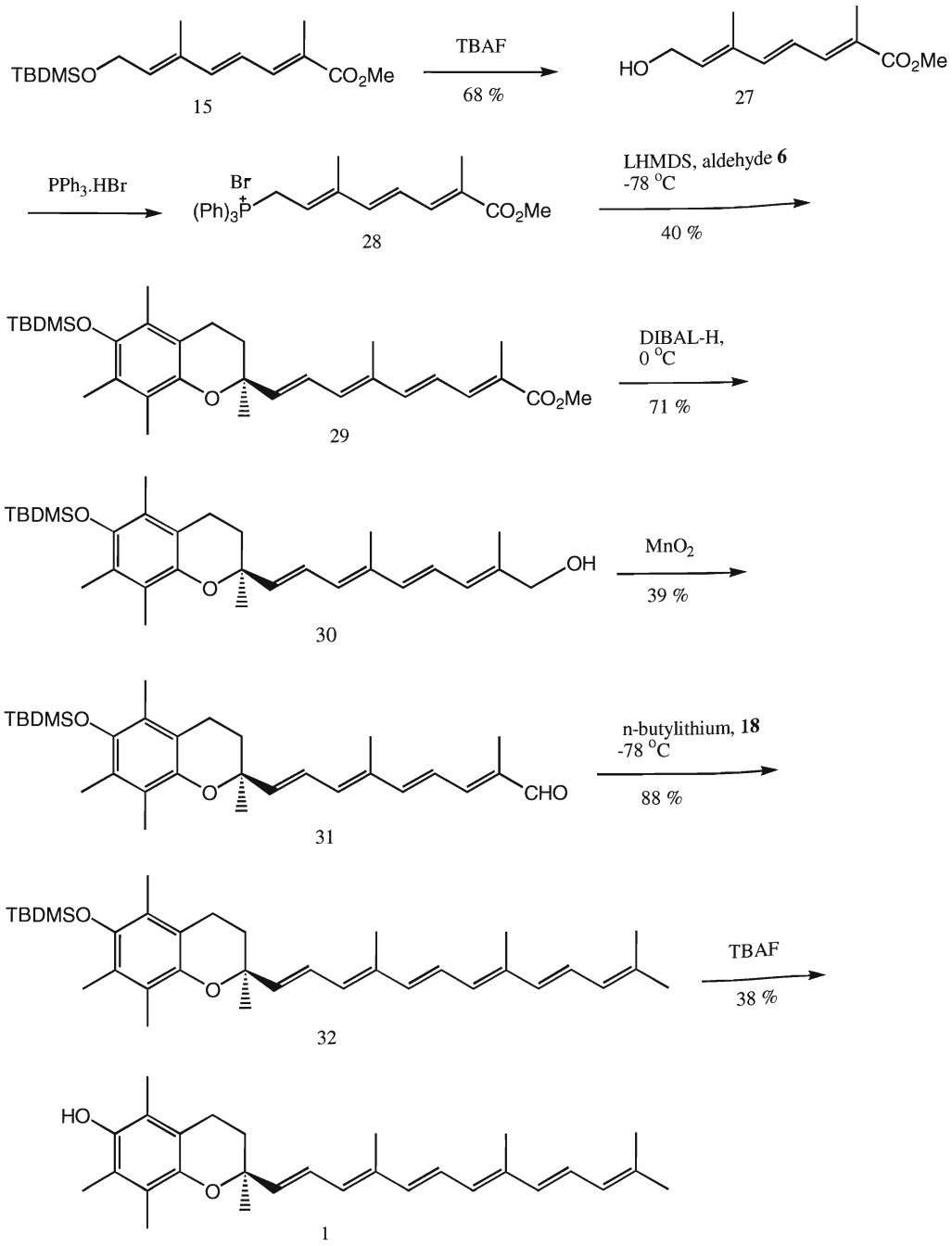


Scheme 10 Synthesis of trolox aldehyde **6**.

The synthesis of aldehyde **6** followed the protocols reported by our group[117] (Scheme 10). First, the carboxylic acid group was converted to its methyl ester **22** by refluxing in DCM/methanol which was catalyzed by *p*-toluenesulfonic acid monohydrate. Protection of the phenol group as a silyl ether was followed by reduction with DIBAL-H at -60 °C to give aldehyde **6** as a white solid. The reaction temperature was carefully controlled to not be higher than -60 ~ -55 °C otherwise most of the starting material would be reduced directly to the alcohol.

The final Wittig reaction took place between aldehyde **31** and Wittig salt **18**. n-Butyl lithium was used to generate the corresponding ylide at 0 °C then aldehyde **31** was added

at -78 °C. Compound **32** was obtained with an excellent yield (87 %). Deprotection of silyl ether by TBAF in THF afforded the final product **1**.



Scheme 11 Synthesis of compound **1** using $C_{14} + C_{10} + C_5$ strategy.

2.3 Fluorescent Characterization of α-Tocohexaenol.

α-Tocohexaenol fluoresces in both ethanol and hexanes, with a maximum absorption at 368 nm in ethanol and 367 nm in hexanes (Figure 10 & 11). The absorption coefficients are 43000 ± 1075 M^{-1} cm^{-1} in ethanol and 28000 ± 700 M^{-1} cm^{-1} in hexane respectively. The maximum emission of α-tocohexaenol is 521 nm with excitation at 366 nm (Figure 12).

The fluorescence intensity decreased significantly in aqueous solution. When the H_2O content reached 90 % (v/v), the fluorescence of α-tocohexaenol showed a 7-fold decrease (Figure 13).

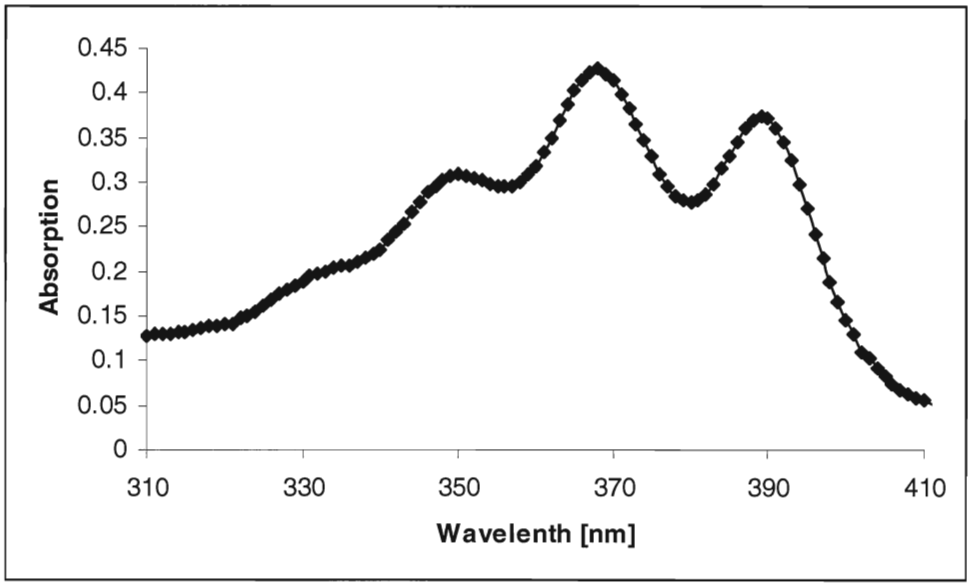


Figure 10. UV-Vis absorption of α-tocohexaenol in ethanol (concentration: 9.2 μM).

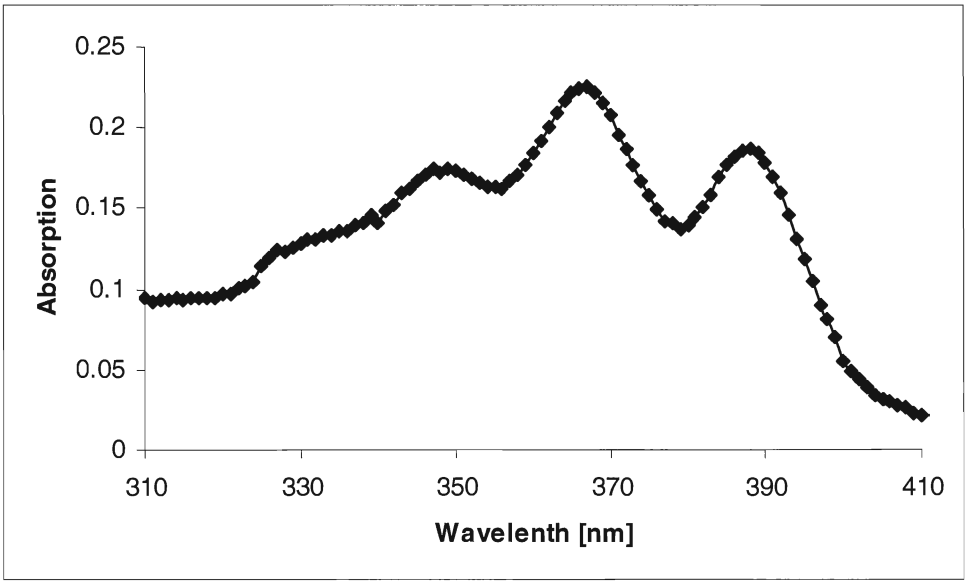


Figure 11. UV-Vis absorption of α-tocohexaenol in hexane (concentration: 9.2 μM).

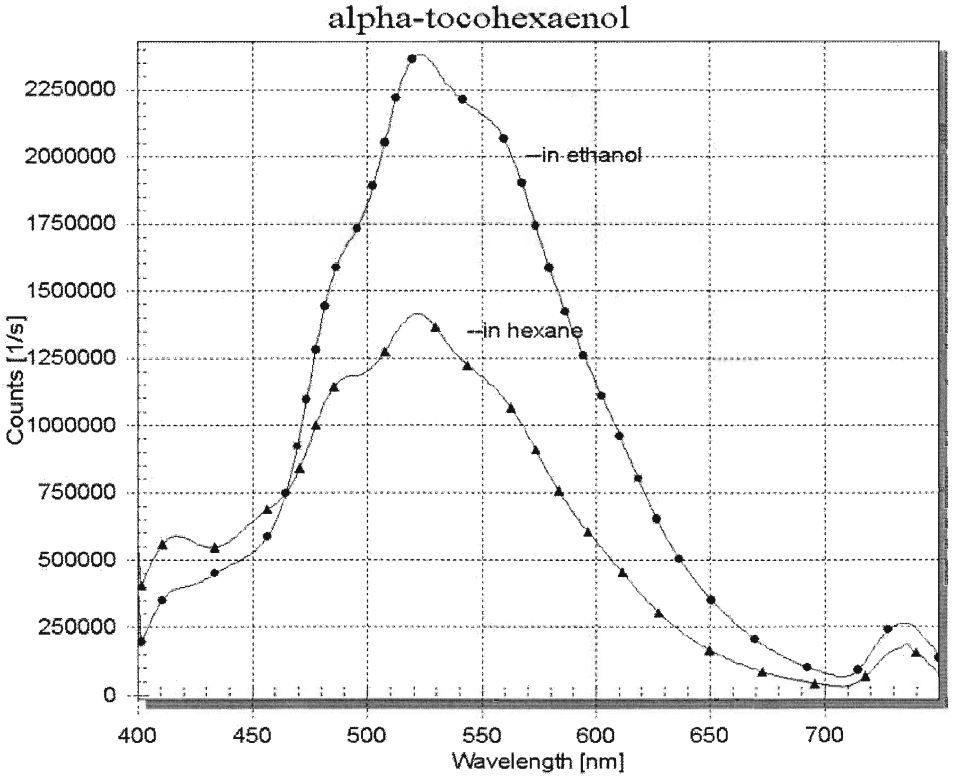


Figure 12. Fluorescence emission of α-tocohexaenol in ethanol & hexane (concentration: 4.6 μM).

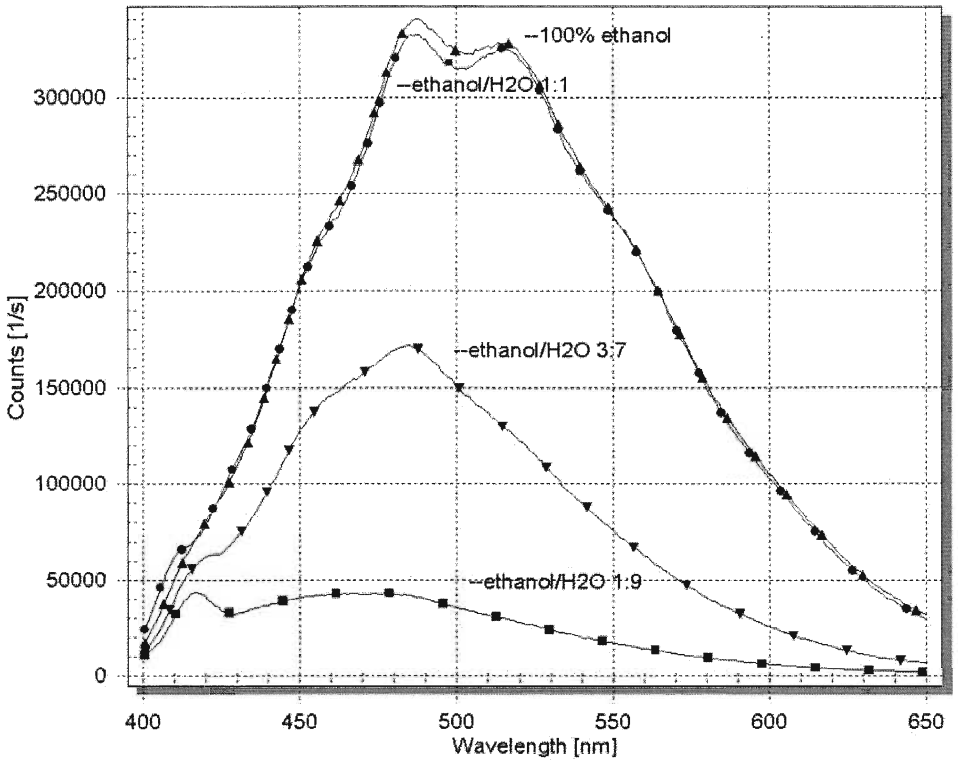


Figure 13. Fluorescence of α-tocohexaenol in aqueous solution.

2.4 Stability Study of α-Tocohexaenol in Solution

Conjugated polyenes such as lycopene[118] are known to be susceptible to temperature, light, and air oxidation. Any future use of α-tocohexaenol as a biological probe for α-tocopherol will require the knowledge of how to handle and store it to preserve its structural integrity.

α-Tocohexaenol can be stored in the dark and at –10 ºC for about one month without any significant changes in its UV absorption spectrum. It is relatively stable in ethanol and hexane if stored at low temperature (0 ºC) and protected from light. The UV-Vis absorption spectrum showed no significant changes with a maximum absorption at 368 nm in ethanol and 367 nm in hexanes after 10 days storage at 0 ˚C and protected from light.

2.4.1 Stability against Air Oxidation

To test the stability against air oxidation, the solutions of α-tocohexaenol (9.2 μM) were prepared in ethanol or hexane, which were then gently bubbled with air for 10 minutes and the UV-Vis absorption spectrum monitored. There were no significant changes of the UV absorption spectrum right after the air bubbling. After the solutions of α-tocohexaenol were put on the bench top overnight at room temperature and protected from light, the UV absorption spectrum of α-tocohexaenol in ethanol stayed the same, but the one in hexane solution changed: its maximum absorption wavelength shifted from 367 nm to366 nm, and the intensity of its maximum absorption decreased from 0.225 at 367 nm to 0.174 at 366 nm (Figure 14). However, no significant changes in either ethanol or hexane solution were observed when checked by TLC after stayed overnight. Finally the TLC showed that there was no α-tocohexaenol left after 24 hours in either ethanol or hexane solution, and many more polar spots could be observed.

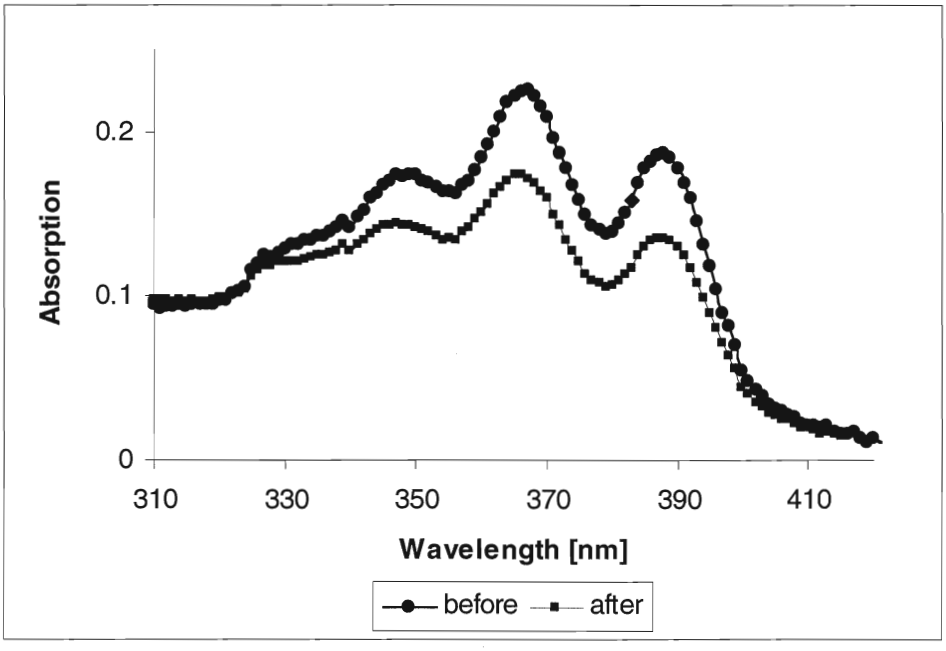


Figure 14. UV-Vis absorption of α-tocohexaenol in hexane after the air was bubbled in.

2.4.2 Stability against UV and Light

To test the stability of α-tocohexaenol against light, the solution of α-tocohexaenol in ethanol was prepared in a glass vial without cap and exposed under a TLC visualization UV lamp (254 nm) for 1 h, and the UV-Vis absorption spectrum was monitored. There was no change in the UV-Vis absorption spectrum. Then the solution of α-tocohexaenol was put on the bench top under room light and the UV-Vis absorption spectrum was monitored again. After 4 hours its maximum absorption wavelength shifted from 368 nm to 366 nm, and its maximum absorption decreased from 0.427 at 368 nm to 0.344 at 366 nm (Figure 17). The TLC showed that little of the starting material (R_f = 0.45, hexane/ethyl acetate 3:1) was left, and some new spots appeared: R_f = 0.58 (minor), R_f = 0.31 (minor), R_f = 0.25 (major), and R_f = 0.19 (minor). It is well known that light can initiate isomerization of polyenes.[118] There are 32 possible *trans-cis* isomers of α-tocohexaenol. Considering that *cis*-isomers are less stable than the all-*trans* isomer, mono-*cis* isomers will dominate after photoisomerization of all-*trans* α-tocohexaenol. Since the *cis*-isomer of an unmethylated double bond is unstable because of the steric interaction on the concave side of the *cis* bend,[119] the main spot on TLC (R_f = 0.25) was probably the 3-*cis* or 7-*cis* isomer. When a *cis* double bond is introduced to an all-*trans* conjugated system such as carotene, the effective conjugation will be decreased and the UV absorption shifts to the blue. [120] This also explains the observed changes in the UV spectrum of α-tocohexaenol after light irradiation (Figure 15).

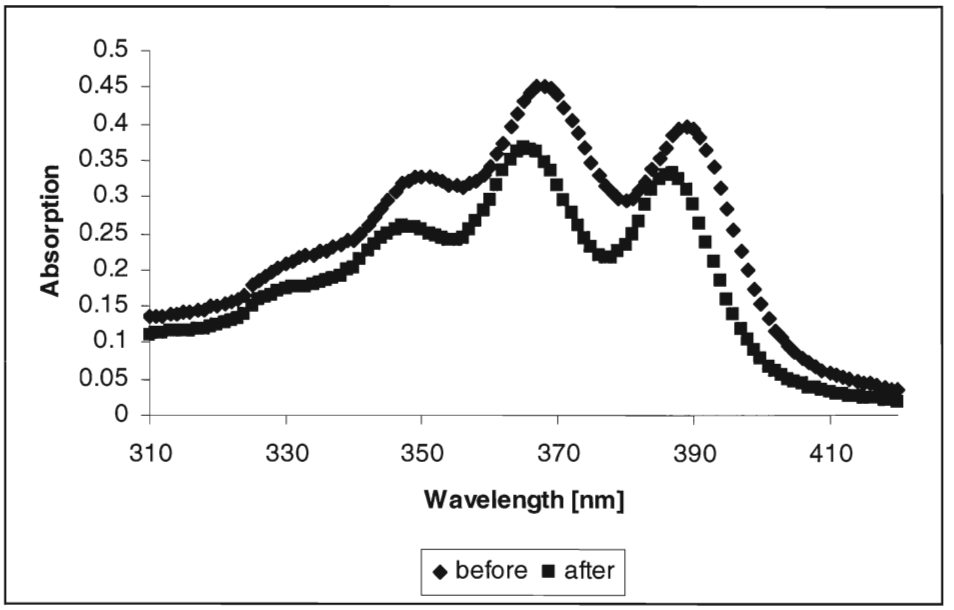


Figure 15. UV-Vis absorption of α-tocohexaenol in ethanol after exposed under room light.

2.4.3 Stability under Acidic Condition

A solution of α-tocohexaenol in ethanol was acidified by *p*-TsOH monohydrate (final concentration of *p*-TsOH monohydrate was 0.042 M) and the UV-Vis absorption spectrum and TLC was monitored. The color of the solution changed very quickly from yellow to pink after the acid was added. The UV-Vis absorption spectrum totally changed 3 hour later (Figure 16), and the TLC showed that there was no α-tocohexaenol left and many more polar spots could be observed.

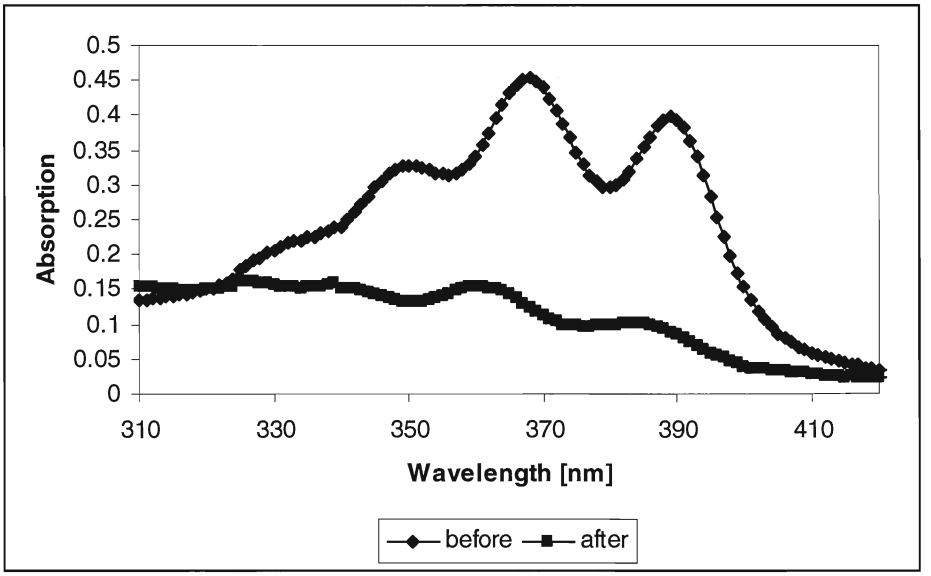


Figure 16. Absorption of α-tocohexaenol in ethanol 3 hours after the solution was acidified by *p*-TsOH.

3. Conclusion and Future Work

The synthesis of α-tocohexaenol, a fluorescent analogue of α-tocopherol, has been successfully completed and reported in this thesis. α-Tocohexaenol has a maximum fluorescence emission at 521 nm when excited at 366 nm and so should not be interfered with by endogenous fluorophores such as trytophan. It is stable in solution for two weeks if stored in the dark at low temperature (0 ˚C). If stored at room temperature and exposed to light, it will decompose within 5 hours. Thus, α-tocohexaenol may be a useful probe in the study of vitamin E if handled appropriately. This analogue has the highest structural similarity to α-tocopherol compared to other fluorescent analogues that have been reported. Upon the successful synthesis of this molecule, the first bioassay would be the measurement of the binding affinity with α-TTP. The new molecule is likely not to show the same affinity as α-tocopherol because it has a more rigid tail; but since the protein binds the vitamin with the chromanol buried and the tail partially escaping the active site,[121] we suspect that it will still bind well enough with α-TTP for use in vitro assay. The next important assay would be the test of its ability as an antioxidant in vitro. We are looking forward to see the results of these assays in the very near future.

4. Experimental

General

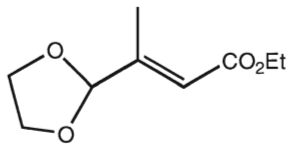
All reagents were purchased from Sigma-Aldrich Chemical Co., Oakville, Ontario. All glassware was dried in an oven of 90 °C overnight, and then cooled in desiccators before use. Cooling baths were prepared with acetone/liquid nitrogen for -78 °C and -60 °C, and ice/water for 0 °C. Air or moisture sensitive reactions were performed under N_2. THF (tetrahydrofuran) was distilled with sodium/benzonephenone immediately before use; benzene was distilled over sodium metal; dichloromethane was distilled over CaH_2; methanol was distilled from Mg/iodine. *n*-Butyllithium was titrated with diphenylacetic acid or N-benzylbenzamide to a yellow or a blue endpoint, respectively, right before use. Reagent grade solvents were used for extractions. Distilled water was used for all aqueous workups.

Analytical thin-layer chromatography (TLC) was performed on Merck 0.25 mm pre-coated silica gel 60 Å F-254 aluminum plates and visualized under UV light, or stained in 5 % $KMnO_4$ solution. Column chromatography was carried out on silica gel (200 – 300 mesh) purchased from Aldrich.

Optical rotations were recorded on a Rudolph Autopol III polarimeter. Fourier transform infrared spectra (FT-IR) were recorded on a Bomem MB-100 spectrometer and the resonance frequency is reported in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker Advance DPX-300 digital FT-NMR spectrometer (300 and 75 MHz, respectively) in $CDCl_3$ with residual chloroform as internal reference (7.281 ppm for ^{1}H and 77.0 ppm for ^{13}C) unless otherwise noted. Chemical shifts are reported as δ values

and coupling constants (*J*) are reported in Hertz (Hz). The following abbreviations are used: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Mass spectra (MS) were recorded on a Carlo Erba/Kratos GC/MS Concept 1S double focusing mass spectrometer interfaced to a Kratos DART acquisition system and a Sun SPARC workstation. Samples were introduced through a direct inlet system. Ions are generated using electron impact (EI) or Fast Atom Bombardment (FAB) and were reported in m/z values. Fluorescence were recorded on a Photon Technologies International QuantaMaster Model QM-2001 L-format, equipped with double-grating monochromators, a 150 W xenon lamp, and Felix 32 software.

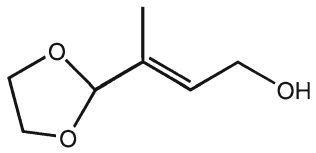
Synthesis of (*E*)-ethyl-3-(1,3-dioxolan-2-yl)but-2-enoate (10)



A mixture of 3-methyl-4-oxo-but-2-enoic acid ethyl ester (14 ml, 92 mmol), ethylene glycol (8.75 ml, 140 mmol), and PPTS (2.6 g, 10 mmol) in benzene (200 ml) was heated to reflux under a Dean-Stark trap for 4 h. Upon cooling, water was added, and the aqueous layer was extracted with ether (3 x 50 ml). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified on SiO_2 (ethyl acetate/hexanes 1:9) to give 16 g (90 %) of colorless oil.

TLC	R_f = 0.4 (hexane/ethyl acetate = 9:1)
^{1}H-NMR	δ 6.03 (s, 1H, =CH), 5.21 (s, 1H, OCHO), 4.20 (q, *J* = 7.2 Hz, 2H, CH_2), 4.00 (m, 4H, OCH_2CH_2O), 2.13 (s, 3H, CH_3), 1.28 (t, *J* = 7.2 Hz, 3H, CH_3).
^{13}C-NMR	δ 166.29, 152.59, 118.50, 105.12, 65.43, 60.00, 14.24, 13.16.
Mass Spectra [EI+]	m/z 186 (M+, 1.3%), 157 (12.2%), 141 (22.3%), 113 (24.3%), 73 (100%).

Synthesis of (*E*)-3-(1,3-dioxolan-2-yl)-but-2-en-1-ol (11)

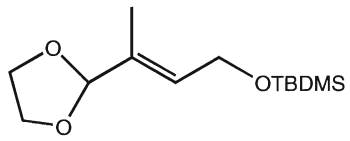


Ester **10** (11.72 g, 63 mmol) in ether (50ml) was added to a solution of $LiAlH_4$ (75.6 ml, 75.6 mmol) in ether (120 ml) at 0 °C. The resulting suspention was stirred for 45 min before being quenched with methanol and H_2O. The aqueous layer was extracted with ethyl ether (4 x 60 ml) and the combined organic layers were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:1) afforded 7.44 g (82 %) of alcohol as colorless oil.

TLC	R_f = 0.27 (hexane/ethyl acetate = 1:1)
^{1}H-NMR	δ 5.86 (t, *J* = 6 Hz, 1H, =CH), 5.13 (s, 1H, CH), 4.26 (d, *J* = 6.3

	Hz, 2H, CH_2O), 4.09 (m, 4H, OCH_2CH_2O), 1.67 (s, 3H, CH_3).
	^{13}C-NMR (75 MHz, $CDCl_3$)
^{13}C-NMR	δ 142.62, 139.22, 119.77, 112.16, 65.41, 63.68, 9.59.
Mass Spectra [EI+]	m/z 143 (M+, 1.7%), 113 (58.8%), 73 (100%), 45 (29.9%)
HRMS	Calcd: 143.07082, found: 143.07125.

Synthesis of (*E*)-3-(1,3-dioxolan-2-yl)-but-2-enyloxy-(*tert*-butyldimethylsilyl)-ether (12)

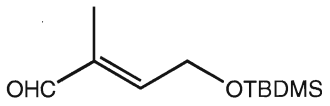


Imidazole (1.43 g, 21 mmol) and tert-butyldimethylsilyl chloride (3.16 g, 21 mmol) were added sequentially to a solution of alcohol **11** (2.9 g, 20 mmol) in DMF (60 ml). After stirring at room temperature for 2 h, water was added and the mixture was extracted with hexanes (4 x 30 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:9) afforded 2.98 g (70 %) of oil.

TLC	R_f = 0.4 (hexane/ethyl acetate = 9:1)
^{1}H-NMR	δ 5.79 (t, *J* = 6 Hz, 1H, =CH), 5.13 (s, 1H, OCHO), 4.29 (d, *J* = 6 Hz, 2H, CH_2O), 4.07 (m, 4H, OCH_2CH_2O), 1.63 (s, 3H, CH_3), 0.88 (s, 9H, $3CH_3$), 0.07 (s, 6H, $2CH_3$).

^{13}C-NMR	δ 132.49, 131.90, 106.89, 65.34, 59.87, 25.92, 18.32, 10.39, -5.18.
Mass Spectra [EI+]	m/z 258 (M+, 0.6%), 83 (100%), 75 (92.3%), 73(39.6%).
HRMS	Calcd: 258.16512, found: 258.16569.

Synthesis of 4-(*tert*-butyldimethylsilanyloxy)-2-methyl-but-2-enal (13)

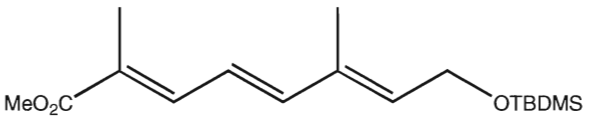


To a solution of compound **12** (2.93 g, 11 mmol) in acetone (120 ml) were added *p*-toluene sulfonic acid monohydrate (211 mg, 1.11 mmol) and water (2.93 ml). The resulting solution was stirred for 10 min. Addition of a cold saturated aqueous $NaHCO_3$ solution was followed by extraction with chloroform (4 x 60 ml). The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:9) afforded 2.28 g (90 %) of oil.

TLC	R_f = 0.4 (hexane/ethyl acetate = 9:1)
^{1}H-NMR	δ 9.44 (s, 1H, CHO), 6.56 (t, *J* = 1.2 Hz, 1H), 4.53 (d, *J* = 6 Hz, 2H, CH_2O), 1.74 (s, 3H, CH_3), 0.94 (s, 9H, $3CH_3$), 0.12 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 194.60, 153.14, 138.00, 60.50, 25.84, 25.65, 9.38, -5.29.

Mass Spectra [EI+] m/z 214 (M+, 2.2%), 171 (10.1%), 157 (16.8%), 75 (100%).

Synthesis of 8-(*tert*-butyldimethylsilanyloxy-2,6-dimethyl-octa-2,4,6-trienoic acid methyl ester (15)



To a solution of a mixture of phosphonate ester **14a** and **14b** (6.75 g, 27 mmol) in dry THF (100 ml) was added LHMDS (27 ml, 1M in THF) at 0 ˚C. After stirring for 30 min, aldehyde **8** (1.89 g, 8.83 mmol) in THF (80 ml) was added dropwise. After 3 h, the reaction was quenched by water and extracted with ethyl acetate (4 x 50 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:9) afforded 2.36 g (86 %) of light yellow oil.

TLC R_f = 0.4 (hexane/ethyl acetate = 9:1)

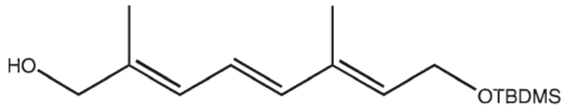
^{1}H-NMR δ 7.15 (d, *J* = 10 Hz, 1H), 6.46 (d, *J* = 1.2 Hz, 3H, CH_3), 1.73 (d, J = 1.2 Hz, 3H, CH_3), 0.83 (s, 9H, $3CH_3$), 0.0 (s, 6H, $2CH_3$).

^{13}C-NMR δ 168.94, 143.67, 138.84, 135.92, 134.05, 126.24, 122.71, 60.39, 51.79, 25.94, 18.38, 12.79, 12.61, -5.14.

Mass Spectra [EI+] m/z 310 (M+, 6.2%), 253 (46.9%), 221 (49.1%), 178 (58.9%), 147 (71.5%), 119 (73.9%), 73 (100%).

HRMS Calcd: 310.19642, found: 310.19487.

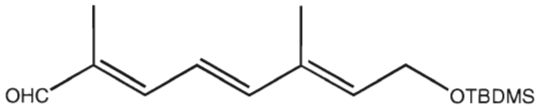
Synthesis of 8-(*tert*-butyldimethylsilanyloxy)-2,6-dimethyl-octa-2,4,6-trien-1-ol (16)



To a solution of ester **15** (1.85 g, 5.96 mmol) in THF (40 ml) was added DIBAL-H (12.5 ml, 1 M solution in THF) at 0 ˚C. After stirring for 2 h, the reaction was quenched with methanol and water, followed by extraction with ethyl acetate (4 x 30 ml). The combined organic extracts were dried over $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:2) afforded 1.60 g (95 %) of oil.

TLC	Rf = 0.4 (hexane/ethyl acetate = 2:1)
^{1}H-NMR	δ 7.4 (m, 1H, =CH), 6.5~6.0 (m, 2H, =CH), 5.5 (t, *J* = 5.2 Hz, 1H, =CH), 4.16 (d, *J* = 6.4 Hz, 2H, CH_2), 3.97 (s, 2H, CH_2), 1.59 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 0.83 (s, 9H, $3CH_3$), 0.0 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 137.05, 137.00, 134.42, 131.95, 125.50, 123.26, 68.66, 60.37, 25.98, 18.40, 14.31, 12.67, -5.09.
Mass Spectra [EI+]	m/z 282 (M+, 1.7%), 133 (18.3%), 93 (21.4%), 75 (100%).
HRMS	Calcd: 282.20151, found: 282.20077.

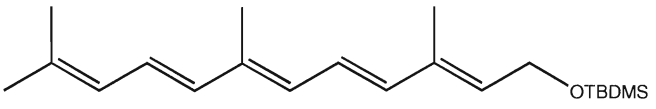
Synthesis of 8-(*tert*-butyldimethylsilanyloxy)-2,6-dimethyl-octa-2,4,6-trienal (17)



A mixture of alcohol **16** (1.82 g, 6.44 mmol), MnO_2 (10.16 g, 116.86 mmol) and Na_2CO_3 (3.42 g, 32.27 mmol) in DCM was stirred overnight at room temperature. After filtration and concentration, the residue was purified on SiO_2 (ethyl acetate/hexanes 1:3) to give 1.50 g (83 %) of yellow oil.

TLC	R_f = 0.48 (hexane/ethyl acetate = 3:1)
^{1}H-NMR	δ 9.36 (s, 1H, CHO), 6.81 (dd, J_1 = 1.2 Hz, J_2 = 10.4 Hz, 1H, =CH), 6.61~6.52 (m, 2H, =CH), 5.77 (t, J = 1.2 Hz, 1H, =CH), 4.29 (d, J = 6 Hz, 2H, OCH_2), 1.79 (d, J = 1.2 Hz, 3H, CH_3), 1.75 (d, J = 1.2 Hz, 3H, CH_3), 0.83 (s, 9H, $3CH_3$), 0.0 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 195.0, 149.3, 145.8, 138.1, 134.1, 122.6, 60.6, 33.6, 26.1, 18.6, 12.8, 9.8, -4.9.
Mass Spectra [EI+]	m/z 280 (M+, 2.4%), 223 (11.7%), 148 (19.8%), 75 (100%).
HRMS	Calcd: 280.18586, found: 280.18541.

Synthesis of 1-(*tert*-butyldimethylsilanyloxy)-3,7,11-trimethyl-dodeca-(2E,4E,6E,8E,10E)-2,4,6,8,10-pentaene (19)

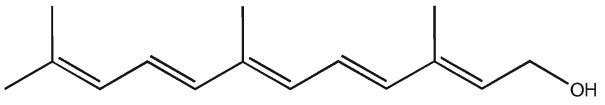


To a suspension of tributyl isoprenyl phosphonium bromide **18** (1.57 g, 4.47 mmol) in dry THF (95 ml) was added n-BuLi (2.8 ml, 4.48 mmol, 1.6 M in THF). After stirring at 0 °C for 30 min under N_2, the solution was cooled to -78 °C. The aldehyde (502 mg, 1.79 mmol) in THF (10 ml) was added dropwise. The reaction was allowed to warm up to room temperature and stirred overnight. It was quenched with water and extracted with ethyl acetate (4 x 40 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:9) afforded 496 mg (83 %) of orange oil.

TLC	R_f = 0.65 (hexane/ethyl acetate = 9:1)
^{1}H-NMR	δ 6.52 (m, 2H, =CH), 6.32 (m, 3H, =CH), 5.96 (d, *J* = 12Hz, 1H, =CH), 5.63 (t, *J* = 6.3 Hz, 1H, =CH), 4.37 (d, *J* = 6 Hz, 2H, OCH_2), 1.96 (s, 3H, CH_3), 1.83 (s, 9H, $3CH_3$), 0.92 (s, 9H, $3CH_3$), 0.09 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 136.83, 135.75, 135.64, 134.93, 134.80, 131.93, 125.97, 124.66, 124.42, 60.44, 26.27, 26.00, 25.98, 18.56, 18.54, 12.84, 12.64, -4.9.

Mass Spectra [EI+]	m/z 332 (M+, 3.1%), 200 (13.1%), 117 (28.1%), 84 (71.6%), 75 (100%).

Synthesis of 3,7,11-trimethyl-dodeca-(2E,4E,6E,8E,10E)-2,4,6,8,10-pentaen-1-ol (9)



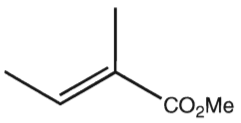
To a solution of compound **19** (355 mg, 1.07 mmol) in THF (50 ml) was added TBAF (10.7 ml, 1 M in THF) at room temperature. After stirring for 4 h, the reaction was quenched with water and extracted with ethyl acetate (4 x 10 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:3) afforded 186 mg (80 %) of orange solid.

TLC	R_f = 0.18 (hexane/ethyl acetate = 3:1)
M.P.	108-110 °C.
^{1}H-NMR	δ 6.61 (m, 2H, =CH), 6.33 (m, 3H, =CH), 5.73 (d, *J* = 11.1 Hz, 1H, =CH), 5.71 (t, *J* = 7.2 Hz, 1H, =CH), 4.31(t, *J* = 6 Hz, 2H, OCH_2), 1.96 (s, 3H, CH_3), 1.88 (s, 3H, CH_3), 1.83 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 136.85, 136.41, 136.25, 135.85, 134.83, 130.68, 130.25, 125.97, 125.23, 124.93, 59.46, 26.26, 18.56, 12.86, 12.61.
Mass Spectra [EI+]	m/z 218 (M+, 16.8%), 200 (42.3%), 145 (42.9%), 105 (46.4%),

91 (51.9%), 43 (100%).

HRMS Calcd: 218.16707, found: 218.16680.

Synthesis of 2-methyl-but-2-enoic acid methyl ester (20)



A mixture of tiglic acid (20.23 g, 0.2 mol), methanol (100 ml) and 100 drops of conc. H_2SO_4 was heated at reflux for 20 h. After adding 100 ml water, it was extracted with DCM (100 ml x 2). The organic layer was washed with dilute $NaHCO_3$ solution, dried over anhydrous $MgSO_4$ and distilled under vacuum to afford 22.6 g (98 %) of colorless oil (56-65 °C/30 mm Hg).

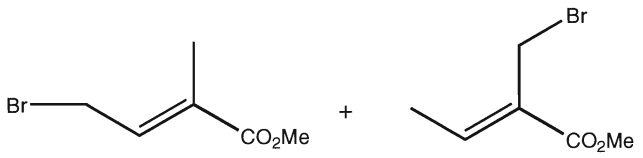
^{1}H-NMR δ 6.90 (dd, J_1 = 7 Hz, J_2 = 2.0 Hz, 1H, =CH), 3.71 (s, 3H, OCH_3), 1.84 (s, 3H, CH_3), 1.81 (d, J = 8 Hz, 3 H, CH_3).

^{13}C-NMR δ 168.60, 137.18, 128.47, 51.61, 14.29, 12.00.

Mass Spectra [EI+] m/z 114(M+, 52.1%), 83(57.3%), 55(100%), 43(53%).

HRMS Calcd: 114.06808, found: 114.06811.

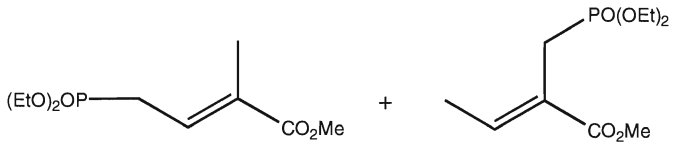
Synthesis of 4-bromo-2-methyl-but-2-enoic acid methyl ester and 2-bromomethyl-but-2-enoic acid methyl ester (21a, 21b)



A mixture of compound **20** (10 g, 87.5 mmol) and NBS (17.25 g, 97.5 mmol) in CCl_4 (100 ml) was heated at reflux for 14 h. After filtration and concentration, it was purified on SiO_2 (DCM) to give 10.1 g (60 %) of a mixture of E and Z isomers (E/Z = 2:1).

TLC	R_f = 0.65 (DCM)
^{1}H-NMR	δ 7.13 (m, *J* = 7.5 Hz, 1H, =CH), 6.94 (m, *J* = 6.8 Hz, 1 H, =CH), 4.25 (s, 2H, CH_2Br), 4.03 (d, *J* = 8.4 Hz, 2H, CH_2Br), 3.81, 3.79 (2s, 6H, $2CH_3$), 1.95 (m, $2CH_3$).
Mass Spectra [EI+]	m/z 193 (M+, 50.1%), 191 (41.5%), 113 (100%), 82 (25.2%), 53 (32.3%)
HRMS	Calcd: 191.97859, found: 191.97845.

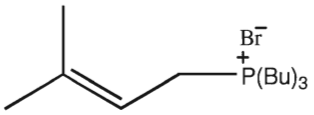
Synthesis of 4-(diethoxy-phosphoryl)-2-methyl-but-2-enoic acid methyl ester (14a, 14b)



A mixture of compound **21a** and **21b** (8 g, 41.2 mmol) and triethylphosphite (7.2 ml, 41.4 mmol) was heated at reflux for 5 h. After concentrated under vacuum, it was purified on SiO_2 (ethyl acetate/methanol 9:1) to give 8.71 g (85 %) of phosphate (E/Z = 3:2).

TLC	R_f = 0.22 (ethyl acetate/methanol = 9:1)
^{1}H-NMR	δ 6.77 (m, 1H, =CH), 6.41 (d, *J* = 5.7 Hz, 1H, =CH), 5.91 (d, *J* = 5.1 Hz, 1H, =CH), 4.17 (m, 4H, $2POCH_2$), 3.78, 3.75 (2s, 6H, $2CH_3$), 2.80 (dd, J_1 = 8.8 Hz, J_2 = 23.4 Hz, PCH2), 3.50~3.34 (dd, J_1 = 8 Hz, J_2 = 24 Hz, 2H, PCH2), 1.94 (d, J = 3.04 Hz, 3H, CH_3), 1.40 (m, $2CH_3$).
Mass Spectra [EI+]	m/z 250 (M+, 42.6%), 190 (36.0%), 162 (50.2%), 109 (57.1%), 81 (100%).
HRMS	Calcd: 250.09701, found: 250.09639.

Synthesis of tributyl-(3-methyl-but-2-enyl)-phosphonium bromide (18)

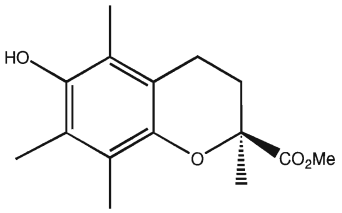


A mixture of tributyl phosphine (2 ml, 17.2mmol) and 4-bromo-2methyl-2-butene (4.25 ml, 17.2 mmol) in toluene (30 ml) was heated at reflux for 5 h. After the toluene was removed by vacuum, hexanes (60 ml) was added and the solution was heated at reflux for

1 h. After cooled to room temperature the solid was filtered and dried under vacuum to give a white crystal (5.48 g, 91 %).

M.P.	61-63 ˚C.
^{1}H-NMR	δ 4.99 (m, =CH), 3.40 (q, *J* = 7.8 Hz, 2H, CH2P), 2..49~2.34 (m, 6H, 3CH3)
^{13}C-NMR	δ 109.08, 108.96, 24.01, 23.81, 23.75, 23.68, 19.19, 18.57, 13.41.
Mass Spectra [FAB]	m/z 271(M-Br, 100%)

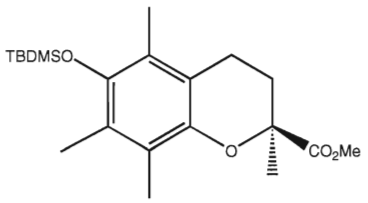
Synthesis of (*S*)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid methyl ester (22)



To a solution of (*S*)-trolox (1.00g, 2.01 mmol) in 100 ml of DCM and methanol (1:1) was added *p*-toluene sulphonic acid (0.40 g, mmol). The resulting solution was heated at reflux for 18 h. Water was added and the aqueous layer was extracted with chloroform (3 x 20 ml). The combined organic layers were washed with water and brine, dried over Mg2SO4, and concentrated. Purification on SiO_2 (DCM/methanol 10:1) afforded 0.94 g white solid (88%).

TLC	R_f = 0.65 (DCM/metahnol = 10:1)
M.P.	134-136 ˚C
^{1}H-NMR	δ 3.69 (s, 3H, OCH_3), 2.65 (m, 2H, CH_2), 2.47 (m, 1H, CH_2), 2.20 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 1.91 (m, 1H, CH_2), 1.62 (s, 3H, CH_3).
^{13}C-NMR	δ 174.47, 145.53, 121.21, 118.36, 116.89, 52.34, 30.61, 25.42, 20.95, 12.20, 11.81, 11.24.
Mass Spectra [EI+]	m/z 264 (M+, 77%), 205 (100%), 164 (76%).

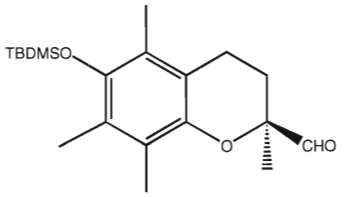
Synthesis of (*S*)-6-(*tert*-butyl dimethyl silyl-oxyl)-2,5,7,8-tetramethyl-chroman-2-carboxylic acid methyl ester (23)



To a solution of compound **22** (493 mg, 1.87 mmol) in anhydrous DMF (5 ml) was added imidazole (515 mg, 7.56 mmol) and TBDMS chloride (570 mg, 3.78 mmol). The mixture was heated to 85 ˚C and stirred under N_2 overnight. After water was added, the mixture was extracted with ethyl acetate (4 x 25 ml). The combined organic layers were washed with water and brine, dried over $MgSO_4$ and concentrated. Purification on SiO_2 (DCM/hexanes 3:1) afforded 700 mg (99 %) of colorless oil.

TLC	R_f = 0.53 (DCM/hexane = 3:1)
^{1}H-NMR	δ 3.68 (s, 3H, OCH_3), 2.58 (m, 2H, CH_2), 2.45 (m, 1H, CH_2), 2.16 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.90 (m, 1H, CH_2), 1.61 (s, 3H, CH_3), 1.08 (s, 9H, $3CH_3$), 0.13 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 174.55, 144.81, 122.70, 117.05, 52.28, 30.57, 26.09, 25.38, 21.05, 18.61, 14.31, 13.37, 11.89, -3.30, -3.36.
Mass Spectra [EI+]	m/z 378 (M+, 8.9%), 189 (24.8%), 147 (100%).

Synthesis of (*S*)-6-(*tert*-butyldimethylsilanyloxy)-2,5,7,8-tetramethyl-chroman-2-carbadehyde (6)

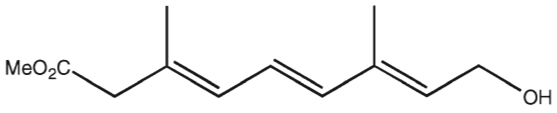


The solution of compound **23** (673 mg, 1.78 mmol) in dry DCM (7.5 ml) was cooled to -60 ˚C. After 20 min DIBAL-H (2.14 ml, 1M in DCM) was added to this stirred solution. The mixture was stirred under N_2 for 2 h. The reaction was quenched with methanol, and then the mixture was poured into water and extracted with ethyl acetate (3 x 30 ml). The combined organic layers were washed with water and brine, dried over anhydrous

$MgSO_4$ and concentrated. Purification on SiO_2 (DCM/hexanes 3:1) afforded 341 mg (55 %) of white crystal.

TLC	R_f = 0.52 (DCM/hexane = 3:1)
M.P.	70-72 ˚C
^{1}H-NMR	δ 9.64 (s, 1H, CHO), 2.56 (m, 2H, CH_2), 2.30 (m, 1H, CH_2), 2.18 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 1.84 (m, 1H, CH_2), 1.40 (s, 3H, CH_3), 1.08 (s, 9H, $3CH_3$), 0.13 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 204.81, 145.56, 145.15, 126.47, 123.86, 122.83, 117.61, 80.26, 27.93, 26.08, 21.59, 20.48, 18.60, 14.34, 13.36, 12.04, -3.31.
Mass Spectra [EI+]	m/z 348 (M+, 42.2%), 319 (33%), 277 (100%), 73 (56.3%).

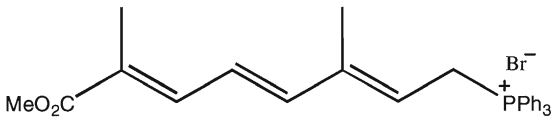
Synthesis of 8-hydroxy-2,6-dimethyl-octa-2,4,6-trienoic acid methyl ester(27)



To a solution of compound **15** (2.23 g, 7.19 mmol) in THF (250 ml) was added TBAF (33 ml, 1 M in THF) under N_2. After 4 h the reaction was quenched with water followed by extraction with ethyl acetate (4 x 50 ml). The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (hexanes/ethyl acetate 1:1) afforded 962 mg (68 %) of pale yellow crystal.

M.P.	58-60 °C.
TLC	R_f = 0.27 (hexane/ethyl acetate = 9:1)
^{1}H-NMR	δ 7.28 (d, *J* = Hz, 1H, =CH), 6.55 (m, 2H, =CH), 5.84 (t, 1H, =CH), 4.37 (d, *J* = 6.6, 2H, CH_2), 3.78 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.87 (s, 3H, CH_3).
^{13}C-NMR	δ 168.62, 143.29, 138.63, 135.92, 134.12, 126.74, 123.45, 59.51, 51.84, 12.82, 12.58.
Mass Spectra [EI+]	m/z 210 (M+, %), 196 (53.3%), 119 (91.7%), 107 (96.3%), 91 (100%), 41 (59.1%).

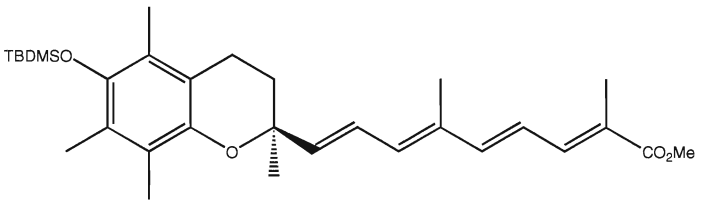
Synthesis of triphenyl-(2,6-dimethyl-octa-2,4,6-trienoic acid methyl ester)-phosphonium bromide(28)



The mixture of alcohol **27** (312 mg, 1.59 mmol) and triphenylphosphinehydrobromide (657 mg, 1.91 mmol) in DCM and ethyl ether (10 ml, 3:2) was stirred for 4 h at room temperature under N_2. After the solvent was removed, the residue was washed with hexanes (5 x 10 ml), dried under vacuum to give 865 mg of orange foam, and it was used directly in next step without further purification.

M.P.	122-124 °C
^{1}H-NMR	δ 7.18 (d, *J* = 9.6, 1H, =CH), 6.4 (m, 2H, =CH), 5.58 (m, 1H, =CH), 5.00 (m, 2H, PCH_2), 3.73 (s, 3H, OCH_3), 1.93 (s, 3H, CH_3), 1.54 (d, *J* = 3.6 Hz, 3H, CH_3).
Mass Spectra [FAB]	m/z 441 (M-Br), 100%), 262(38.3%), 55(58%).
HRMS	Calcd: 441.19834, found: 441.20047.

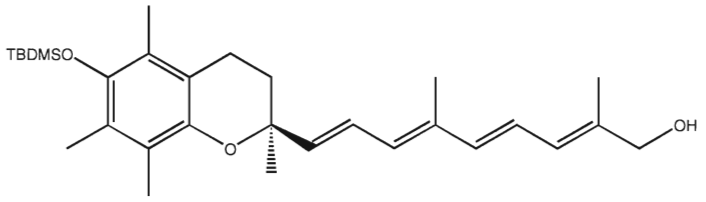
Synthesis of (*S*)-6-(*tert*-butyldimethylsilanyloxy)-2,5,7,8-tetramethyl- chroman-2(4,8-dimethyl-1,3,5,7-tetraenoic acid methyl ester) (29)



To a solution of phosphonium bromide **28** (410 mg, 0.79 mmol) in THF (10 ml) was added LHMDS (0.27 ml, 0.27 mmol) at 0 °C under N_2. The resulting red wine-like solution was stirred for 30 min, and was cooled to – 78 °C. The Trolox aldehyde **6** (92 mg, 0.26 mmol) in THF (2 ml) was added through a syringe. The mixture was allowed to warm up to room temperature and stirred overnight. Water was added followed by extraction with ethyl acetate (4 x 20 ml). The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (DCM/hexanes 3:1) afforded 55.6 mg (40 %) of light yellow oil.

TLC	R_f = 0.42 (DCM/hexane = 3:1)
^{1}H-NMR	δ 7.29 (d, *J* = 12 Hz, 1H, =CH), 7.09 (d, *J* = 12 Hz, 1H, =CH), 6.60 (m, 2H, =CH), 6.34 (t, *J*=12, 1H, =CH), 5.59 (d, *J*=12 Hz, 1H, =CH), 3.80 (s, 3H, CH_3), 2.60 (m, 2H, CH_2), 2.58 (m, 1H, CH_2), 2.19 (s, 3H, Ar-CH_3), 2.11 (s, 3H, Ar-CH_3),, 2.05 (s, 3H, Ar-CH_3), 2.01 (s, 3H, CH_3), 1.98 (m, 1H, CH_2), 1.88 (s, 3H, CH_3), 1.56 (s, 3H, CH_3), 1.05 (s, 9H, $3CH_3$), 0.13 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 168.99, 145.73, 144.47, 144.44, 139.04, 137.25, 135.89, 130.73, 126.11, 125.69, 123.59, 123.24, 122.51, 117.68, 75.98, 51.81, 34.76, 33.73, 27.51, 25.29, 21.24, 18.60, 14.38, 13.43, 12.84, 12.44, 11.88, -3.31.
Mass Spectra [EI+]	m/z 510 (M+, 23.9%), 378 (23.1%), 319 (58.4%), 279 (42.3%), 73 (100%).
HRMS	Calcd: 510.31645, found: 510.31621.

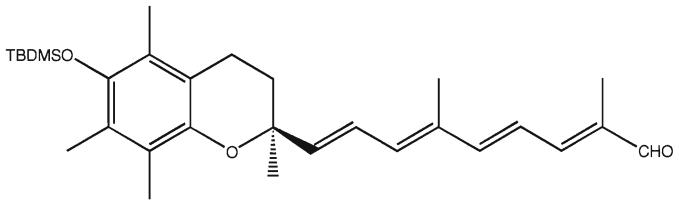
Synthesis of (2*E*,4*E*,6*E*,8*E*)-9-(6-(*tert*-butyldimethylsilanyloxy)-(*S*)-2,5,7,8-tetramethylchroman-2-yl)-2,6-dimethylnona-2,4,6,8-tetraen-1-ol) (30)



To a solution of ester **17** (270 mg, 0.53 mmol) in THF was added DIBAL-H (1.6 ml, 1M in DCM) at 0 °C. The resulting solution was stirred for 2 h under N_2. The reaction was quenched with methanol and water, followed by extraction with ethyl acetate (4 x 20 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:3) afforded 180 mg (71 %) of oil.

TLC	R_f = 0.33 (hexane/ethyl acetate = 3:1)
^{1}H-NMR	δ 6.91 (d, *J*=12, 1H, =CH), 6.49~6.28 (m, 3H, =CH), 6.20 (d, *J*=10.8, 1H, =CH), 5.49 (d, *J*=12, 1H, =CH), 4.13 (m, 2H, OH-CH_2), 2.60 (t, 2H, CH_2), 2.18 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 1.98~1.85 (m, 8H, CH_2 and $2CH_3$), 1.55 (s, 3H, CH_3), 1.05 (s, 9H, $3CH_3$), 0.13 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 136.30, 135.29, 127.66, 127.40, 125.92, 125.76, 122.57, 76.58, 69.40, 68.67, 63.18, 33.71, 27.83, 27.47, 27.05, 26.09, 21.26, 21.06, 20.44, 20.37, 18.60, 14.32, 12.36, 12.01, -3.32, -3.37.
Mass Spectra [EI+]	m/z 482(M+, 4.05%), 86(81%), 84(100%).
HRMS	Calcd: 482.32162, found: 482.30629.

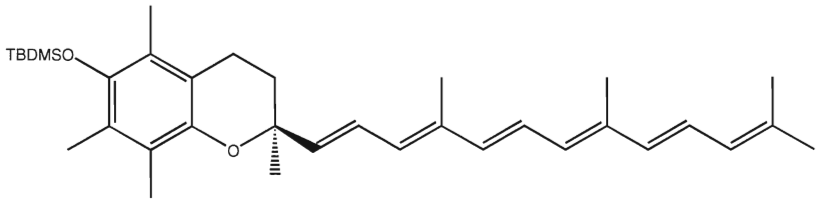
Synthesis of (2*E*,4*E*,6*E*,8*E*)-9-(6-(*tert*-butyldimethylsilanyloxy)-(*S*)-2,5,7,8-tetramethylchroman-2-yl)-2,6-dimethylnona-2,4,6,8-tetraenal) (31)



A mixture of alcohol **30** (180 mg, 0.38 mmol), MnO_2 (600 mg, 6.9 mmol) and Na_2CO_3 (200 mg, 1.89 mmol) in DCM (15 ml) was stirred for 3 h at room temperature. After filtration and concentration, the residue was purified on SiO_2 (ethyl acetate/hexanes 1:3) to give 69 mg (39 %) of yellow oil.

TLC	R_f = 0.58 (hexane/ethyl acetate = 3:1)
^{1}H-NMR	δ 9.49 (s, 1H, CHO), 7.18 (d, *J* = 12 Hz, 1H, =CH), 6.97 (d, *J* = 9.6 Hz, 1H, =CH), 6.72~6.65 (m, 2H, =CH), 6.40~6.32 (t, *J* = 12 Hz, 1H, =CH), 5.64 (d, *J* = 12 Hz, 1H, =CH), 2.60 (t, *J* = Hz, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.98~1.85 (m, 8H, CH_2 and $2CH_3$), 1.56 (s, 3H, CH_3), 1.05 (s, 9H, $3CH_3$), 0.13 (s, 6H, $2CH_3$).
^{13}C-NMR	δ 193.61, 148.21, 145.37, 144.66, 143.45, 137.21, 135.73, 135.98, 134.70, 131.88, 124.53, 122.67, 121.82, 121.33, 116.65, 75.06, 59.35, 32.69, 26.46, 25.06, 20.20, 17.57, 13.37, 12.41, 11.42, 10.84, 8.61, -4.33.
Mass Spectra [EI+]	m/z 480 (M+, 21.6%), 221 (27.9%), 149 (39.7%), 95 (55.2%), 73 (100%).
HRMS	Calcd: 480.30597, found: 480.30605.

Synthesis of *tert*-butyldimethylsilanyloxy ((*S*)-2,5,7,8-tetramethyl-2-((1*E*,3*E*,5*E*,7*E*,9*E*)-4,8,12-tetramethyltrideca-1,3,5,7,9,11-hexaenyl)chroman-6-yoxyl)silane (32)

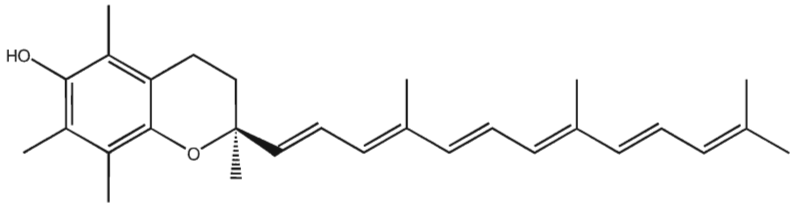


To a solution of phosphonium salt **13** (160 mg, 0.46 mmol) in THF (12 ml) was added n-BuLi (0.33 ml, 1.39 M in hexane) at 0 ˚C. The resulting pale yellow solution was stirred for 30 min under N_2. The aldehyde **20** (81.7 mg, 0.17 mmol) in THF (2 ml) was added after the reaction mixture was cooled to -78 ˚C. The mixture was allowed to warm up to room temperature and stirred overnight. Water was added followed by extraction with ethyl ether (4 x 15 ml). The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:9) afforded 79.3 mg (88 %) of orange oil.

TLC	R_f = 0.67 (hexane/ethyl acetate = 9:1)
^{1}H-NMR	δ 6.93 (d, *J*=12, 1H, =CH), 6.60~6.18 (m, 6H, =CH), 5.99 (d, *J*=10.8, 1H, =CH), 5.5(d, *J*=12, 1H, =CH), 2.60 (t, 2H, CH_2), 2.20 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 1.98~1.85 (m, 14H, $1CH_2$ and $3CH_3$), 1.57 (s, 3H, CH_3), 1.06 (s, 9H, $3CH_3$), 0.14 (s, 6H, $2CH_3$).

^{13}C-NMR	δ ppm 145.88, 144.37, 137.70, 136.02, 135.74, 135.19, 134.99, 131.29, 127.44, 126.07, 125.11, 124.80, 123.54, 122.62, 117.80, 75.96, 33.74, 27.52, 26.29, 26.12, 21.31, 18.62, 18.59, 14.38, 13.44, 12.90, 12.38, 11.99, -3.30.
Mass Spectra [EI+]	m/z 532 (M+, 9.5%), 319 (39.3%), 279 (30.5%), 221 (24.9%), 91 (44.5%), 73 (100%), 57 (50.2%), 43 (83.8%).
HRMS	Calcd: 532.37366, found: 5323.37321.

Synthesis of (*S*)-6-hydroxy-2,5,7,8-tetramethyl-2-((1*E*,3*E*,5*E*,7*E*,9*E*)-4,8,12-tetramethyl-trideca-1,3,5,7,9,11-hexaenyl)chromane (1)



To a solution of compound **32** (50 mg, 0.09 mmol) in THF (5 ml) was added TBAF (0.2 ml, 1 M in THF) at room temperature under N_2. After stirring for 2 h, the reaction was quenched with water and extracted with ethyl acetate (4 x 10 ml). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated. Purification on SiO_2 (ethyl acetate/hexanes 1:3) afforded 14.3 mg (38 %) of compound **1** as an orange oil.

TLC	R_f = 0.45 (hexane/ethyl acetate = 3:1)
^{1}H-NMR	δ 6.92 (d, *J*=12, 1H, =CH), 6.63~6.22 (m, 6H, =CH), 5.97 (d,

	J=10.8, 1H, =CH), 5.49(d, *J*=12, 1H, =CH), 4.19 (b, 1H), 2.65 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 1.98~1.85 (m, 14H, $1CH_2$ and $3CH_3$), 1.57 (s, 3H, CH_3), 0.93 (s, 9H, $3CH_3$), 0.12 (s, 6H, $2CH_3$).
^{13}C-NMR	δ ppm 145.47, 144.84, 137.62, 136.92, 136.10, 135.81, 134.94, 131.21, 127.25, 126.33 126.03, 125.19, 124.84, 121.13, 118.52, 117.65, 75.89, 34.66, 33.74, 31.58, 27.59, 26.27, 25.27, 21.16, 12.88, 12.23, 12.16, 12.00, 11.29.
Mass Spectra [EI+]	m/z 418(M+, 1.6%), 205(3.8%), 149(13.6%), 75(100%).
HRMS	calcd 418.28718, found 418.28669.

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